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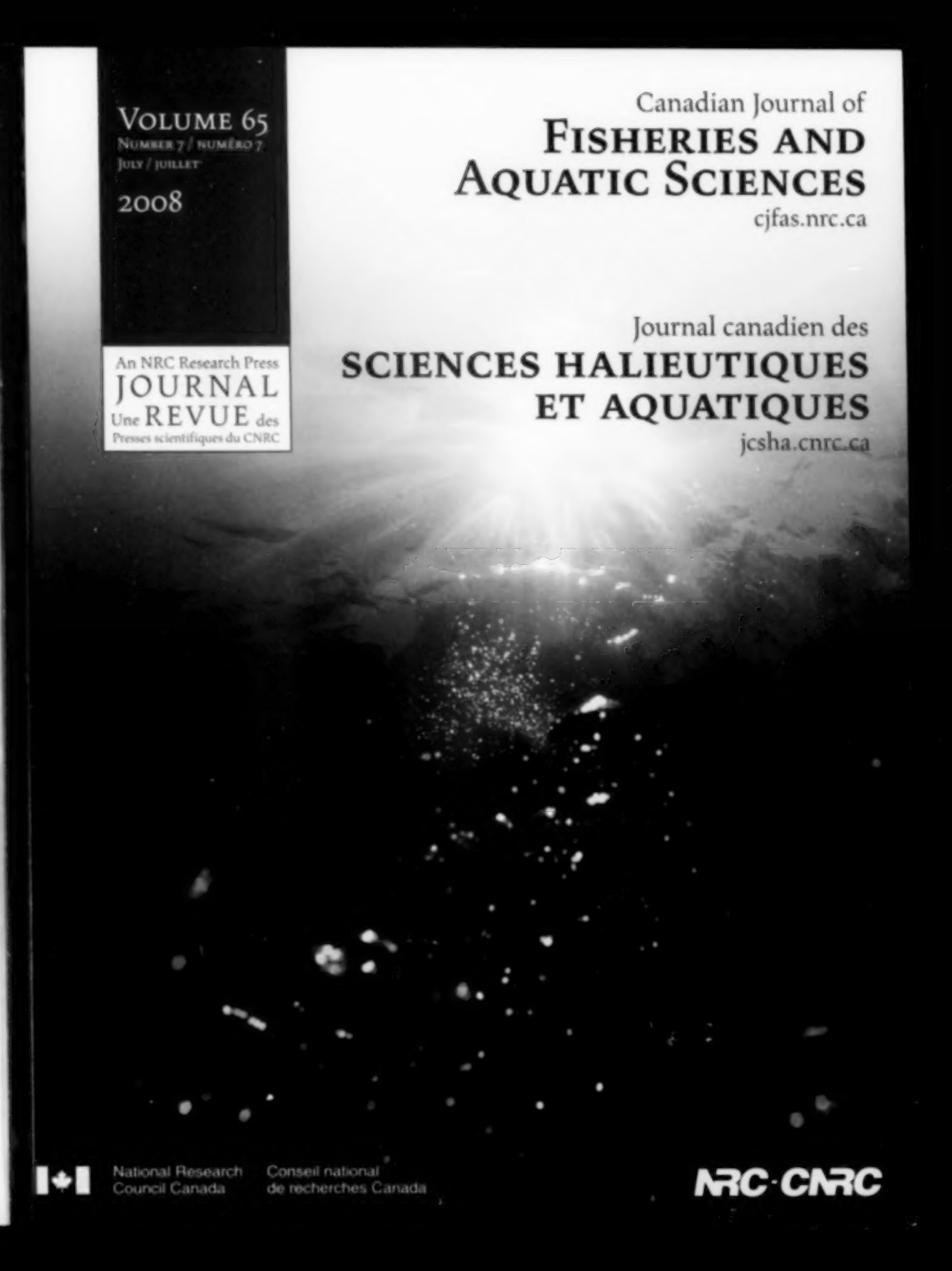

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RAPID COMMUNICATION / COMMUNICATION RAPIDE

Utilizing magnetic resonance imaging (MRI) to assess the effects of angling-induced barotrauma on rockfish (*Sebastes*)

Bonnie L. Rogers, Christopher G. Lowe, Esteban Fernández-Juricic, and Lawrence R. Frank

Abstract: The physical consequences of barotrauma on the economically important rockfish (*Sebastes*) were evaluated with a novel method using T_2-weighted magnetic resonance imaging (MRI) in combination with image segmentation and analysis. For this pilot study, two fishes were captured on hook-and-line from 100 m, euthanized, and scanned in a 3 Tesla human MRI scanner. Analyses were made on each fish, one exhibiting swim bladder overinflation and exophthalmia and the other showing low to moderate swim bladder overinflation. Air space volumes in the body were quantified using image segmentation techniques that allow definition of individual anatomical regions in the three-dimensional MRIs. The individual exhibiting the most severe signs of barotrauma revealed the first observation of a gas-filled orbital space behind the eyes, which was not observable by gross dissection. Severe exophthalmia resulted in extreme stretching of the optic nerves, which was clearly validated with dissections and not seen in the other individual. Expanding gas from swim bladder overinflation must leak from the swim bladder, rupture the peritoneum, and enter the cranium. This MRI method of evaluating rockfish following rapid decompression is useful for quantifying the magnitude of internal barotrauma associated with decompression and complementing studies on the effects of capture and discard mortality of rockfishes.

Résumé : Nous avons évalué les conséquences physiques des barotraumatismes sur les sébastes du genre *Sebastes*, des poissons d'importance économique, à l'aide d'une méthode inédite utilisant l'imagerie par résonance magnétique (MRI) pondérée en T_2 en combinaison avec la segmentation et l'analyse des images. Dans cette étude pilote, nous avons capturé deux poissons à la ligne à une profondeur de 100 m et les avons euthanasiés et analysés dans un scanner à 3 teslas pour humains. Les analyses faites chez les deux poissons montrent, chez l'un, une inflation excessive de la vessie natatoire et une exophtalmie et, chez l'autre, une inflation excessive faible à modérée de la vessie natatoire. Nous avons mesuré les volumes des espaces aériens dans le corps par des techniques de segmentation des images qui permettent une définition des diverses régions anatomiques en MRI 3D. L'individu qui présente les symptômes les plus importants de barotraumatisme possède un espace orbital rempli de gaz derrière les yeux qui n'est pas visible à la dissection grossière. L'exophtalmie sévère cause un étirement extrême des nerfs optiques qui se vérifie clairement à la dissection et qui n'est pas visible chez l'autre individu. Le gaz en expansion provenant de l'inflation excessive de la vessie natatoire doit s'échapper de la vessie, fendre le péritoine et s'infiltrer dans le crâne. Notre méthode d'évaluation des sébastes par MRI après une décompression rapide est utile pour mesurer l'importance du barotraumatisme interne associé à la décompression et elle sert de complément aux études des effets de la capture et de la mortalité reliée à la remise à l'eau chez les sébastes.

[Traduit par la Rédaction]

Introduction

In the eastern Pacific, rockfish (*Sebastes* spp.) comprise one of the most important and economically valuable groundfish. Many rockfish populations are declining as a result of high discard mortality and overexploitation (Davis 2002; Parker et al. 2006). High discard mortality of physoclistic fishes has been attributed to barotrauma on the fish resulting in swim bladder overinflation and rupture,

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exophthalmia (eye protrusion), and prolapsed stomach and (or) cloacae (Wilson and Burns 1996; Nichol and Chilton 2006; Hannah and Matteson 2007). The number and degree of visible trauma symptoms in rockfish (*Sebastes* spp.) are highly variable among individuals and species and not a good predictor of survival (Jarvis and Lowe 2008). Knowledge of internal damage associated with barotrauma is limited, and more empirical evidence quantifying morphological and physical damage is needed. Thus far, methods quantifying damage in rockfish have been limited purely to visual inspection.

In red snapper (*Lutjanus campechnus*), two-dimensional X rays revealed a pattern of increasing organ displacement following decompression (Rummer and Bennett 2005), but volumes were estimated by measuring organ dimensions, which is subject to error. Magnetic resonance imaging (MRI), on the other hand, provides a fully three-dimensional (3D) digital image from which any fish organ dimension can be measured accurately (in cubic millimetres). Although X rays create contrast based on bone density, MRI has an advantage of visualizing and depicting soft tissues based on water content. For example, T_1-weighted MRI scanning on the shortfin mako shark (*Isurus oxyrinchus*) and the salmon shark (*Lamna ditropis*) allowed quantification of red muscle that provided precise and unique detail about the position, volume, and types of red muscle (Perry et al. 2007). MRI methodology can reveal internal tissue and organ placement, and displacement, immediately following trauma and specifically display which areas are composed of tissue or gas.

The goals of this study using MRI were (*i*) to characterize the morphological changes of the internal organs and ocular-orbital regions in rapidly decompressed rockfish using MRI techniques and (*ii*) to determine whether this technique could be used as a diagnostic tool for assessing and characterizing internal injury resulting from fishing-related barotrauma. Because of the high cost of MRI operations, only two rockfish were surveyed as part of this "proof of concept" study. We quantified the relative magnitude of differences between a fish exhibiting many signs of internal barotrauma and one showing few signs.

Materials and methods

Two rockfish, one honeycomb (*Sebastes umbrosus*) and one greenblotched (*Sebastes rosenblatti*), were caught using hook-and-line off the coast of Long Beach, California (Potter's Reef) from a depth of 100 m at seafloor, euthanized, and transported in an ice-bath slurry to the MRI facility. The two species have very similar body morphologies, belong to overlapping and homologous ecotypes, and inhabit boulder fields or other rocky bottoms (Love et al. 2002). Although there are likely interspecific differences in morphology and physiology, the goals of this study were to determine whether MRI techniques could be used to quantify internal morphological damage and volume spaces caused by barotrauma.

The honeycomb rockfish (fish with visible trauma (fish VT); 18 cm standard length (SL)) showed visible external signs of barotrauma, including exophthalmia and corneal gas in both eyes, stomach eversion, and an overinflated swim bladder evident by an extremely swollen abdomen. The greenblotched rockfish (fish with no visible trauma (fish NVT); 22 cm SL) showed little external signs of barotrauma, no visible eye trauma, and an overinflated swim bladder visible by a moderately swollen abdomen, although this fish was also rapidly decompressed. Four hours after being caught, both fish were euthanized with an overdose of tricaine methanesulfonate (MS 222) and placed one at a time inside a magnetic resonance imaging (MRI) unit for whole-body scanning. Photographs taken of the fish just after capture and again after MRI imagining indicated little change in physical appearance.

Images were acquired on a 3 Tesla (3T) GE SIGNA clinical imaging system using an eight-channel head array coil (MRI Devices, Waukesha, Wisconsin). Two data image sets were acquired for each specimen, a T_1-weighted sequence and a T_2-weighted sequence. Total imaging time was 2 h for both fish (30 min·scan^{-1}). The T_1-weighted sequence of a fast RF-spoiled gradient recalled echo (FSPGR) produces high contrast between different tissue types, whereas the T_2-weighted sequence was a steady-state free precession (SSFP) method that produces high contrast between tissues and fluids. The T_2-weighted images produced better contrast near the eye regions and were the only scans used for the image analyses in this study.

Three-dimensional image segmentation and subsequent volume measurement of anatomical regions were performed using ITK-SNAP v.1.4.1 (Insight Segmentation and Registration Toolkit) (www.itksnap.org/). Segmentation was performed in a semi-automated fashion whereby regions of interest were determined by an expert user who defined points for the full 3D segmentation. A region-growing algorithm was then used to fill out the full organ volume composed of individual known volume units called voxels. MRI image segmentation is always limited by a well-known effect called "partial voluming" that reduces the contrast at tissue boundaries because multiple tissue types inhabit individual voxels.

Both fish were dissected 2 h after scanning to reveal the organ orientation and the orbital eye regions. All external signs of barotrauma remained virtually unchanged from the time of capture to dissection, probably because fish were euthanized with MS-222 and placed in an ice bath during transport to prevent any further major physiological processes from occurring after capture. The swim bladder and internal organs were made visible by cutting away a section of skin and muscle on the left side of each fish (Fig. 1). The eye orbits and optic nerves were made visible by cutting away the frontal cranial bone and clearing away all surrounding tissue. Optic nerve lengths were measured using digital calipers.

Results

Each MRI cross section of the 3D image set was viewed in a separate window, but all sections were analyzed as a whole. Fish VT (male) showed visible stomach eversion to the right side of the buccal cavity up to the buccal edge (Fig. 1). The stomach edges of fish VT were well defined, with a volume of 13.4 cm^3, or 9.6% of the whole-fish body volume (Fig. 2). The swim bladder volume of fish VT was

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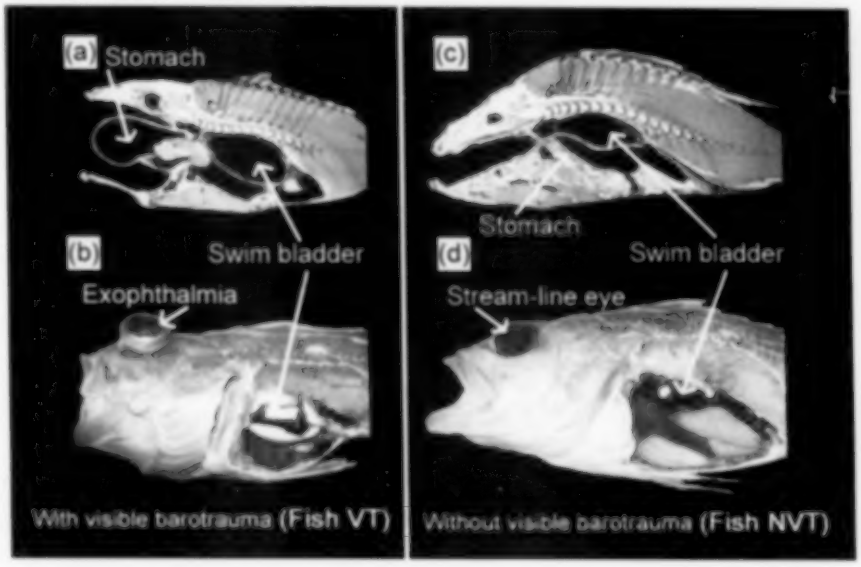
Fig. 1. (*a*) A sagittal view of one magnetic resonance imaging (MRI) slice showing organ displacement in fish with visible trauma (fish VT) resulting from barotrauma. (*b*) Photograph of fish VT with dissection window exposing left side of body cavity. (*c*) Sagittal view of one MRI slice showing less organ displacement in fish with no visible trauma (fish NVT) following rapid decompression. The stomach of fish NVT is small and indistinguishable in this MRI image. (*d*) Photograph of fish NVT with dissection window exposing left side of body cavity. The swim bladder is covered with dark peritoneum lining and is less visible in this photograph than in (*b*). A colour version of this figure is available in the Web version of the Journal.



12.2 cm^3, or 8.7% of the body. The stomach of fish NVT (female) was 1.4 cm^3, or 0.6% of the body (Fig. 2). The swim bladder volume of fish NVT was 10.0 cm^3, or 4.1% of the body. When the left sides of each fish were cut away after imaging to reveal internal organs, the internal organs of fish VT were anteriorly displaced, whereas those observed in fish NVT were not (Fig. 2).

Fish VT showed extreme eye displacement away from its natural medial positioning in the orbital sockets (Fig. 2). MRI images indicate that the eye was physically displaced from the eye socket by subcutaneous gas that formed as an inflated space behind the eye. The volume of this inflated orbital space behind both the right and left eyes combined was 4.9 cm^3, or 3.5% of the body volume. The subcutaneous gas volume behind the left eye of fish VT was visibly greater than the gas volume behind the right eye. Additionally, in fish VT, both the optic nerves of each eye and some ocular muscles were stretched when the eye was displaced as a result of the exophthalmia (Fig. 2). Optic nerve lengths measured from dissections for the right and left eyes of fish VT were 10 mm and 9 mm, respectively, and those of fish NVT were 5 mm and 6 mm, respectively. In fish NVT, no orbital space was visible behind the left eye, but a small orbital space behind the right eye occupied 0.05 cm^3, or 0.02% of the body (Fig. 2). When all tissues were cleared away around the eyes and optic nerves during dissections, the eyes of fish NVT fell slack away from the orbit as a result of optic nerve stretching. The eyes of fish NVT appeared to be streamline in their natural position and the optic nerves were taut and rigid.

Discussion

The high-resolution 3D images of soft tissue acquired with MRI allow the visual depiction and quantitative assessment of the type and degree of barotrauma, including displacement of the stomach, eyes, and other internal organs, optic nerve stretching, swim bladder overinflation, and the inflation of an orbital space behind the eyes. Because both fish in this study were exposed to barotrauma, future studies should compare rapidly decompressed fish with a control fish that is either caught in shallow water to prevent decompression effects or that has been allowed to fully off-gas the swim bladder during ascent. External signs of barotrauma differed between the two species, even though they occupy the same habitats and have very similar morphological characteristics. Jarvis and Lowe (2008) found that external signs of barotrauma varied equally within and among species. Therefore, the differences in response to barotrauma in our study may not necessarily be due to species-specific characteristics. The fish in this study were of opposite sex, and their varying degrees of barotrauma could possibly be due to sex-related differences. The result of barotrauma in gravid females may change the degree of organ displacement or lead to increased internal compression of organs.

Following rapid decompression, swim bladder gases expand due to a decrease in hydrostatic pressure, and an increase in such volumes can reduce body cavity space available to other internal organs (Rummer and Bennett 2005). Dissection of fish VT revealed an enlarged swim bladder, which displaced the stomach, liver, and other

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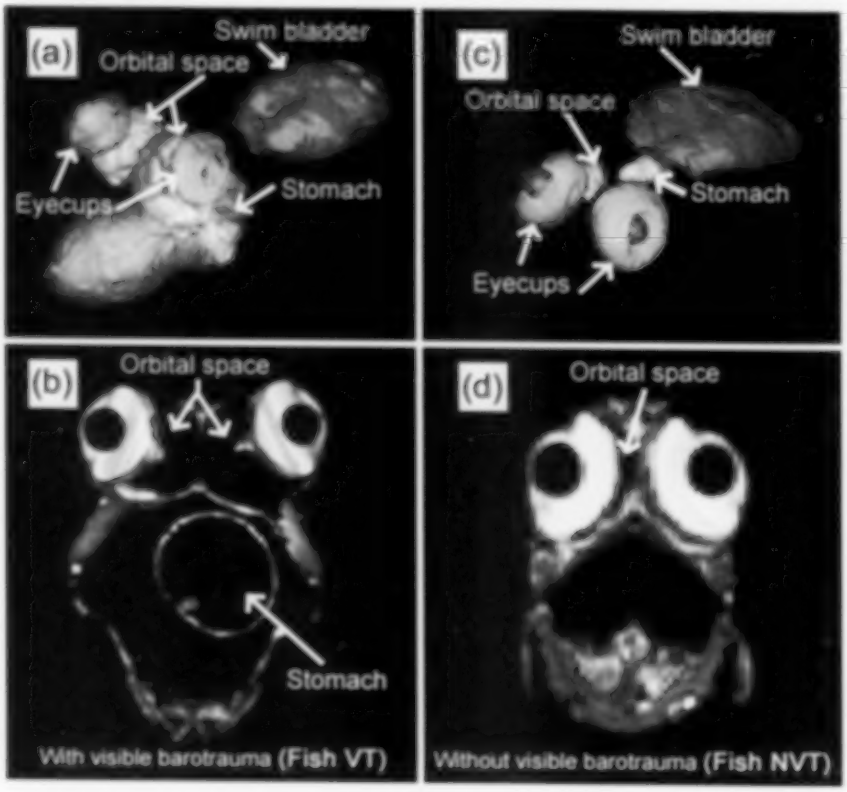
Fig. 2. (*a*) A three-dimensional (3D) rendering of the segmented regions in fish with visible trauma (fish VT), showing the eyecups, stomach, swim bladder, and orbital space. Volumes of these regions were estimated based on magnetic resonance imaging (MRI) voxels. (*b*) Cross-sectional MRI slice of fish VT through the head showing extreme eye displacement. The white protrusions extending off the medial part of the eyes are the optic nerves, which appear short because of their angle relative to this MRI slice. The stomach is prolapsed out the buccal cavity of fish VT. The white circle is the outline of the stomach. (*c*) A 3D rendering of the segmented regions in fish with no visible trauma (fish NVT). (*d*) The eyes of fish NVT exhibit more normal placement, where only a small black region depicts a smaller gas-filled orbital space. A colour version of this figure is available in the Web version of the Journal. In the Web version, in parts *a* and *c*, the eyes are indicated in green, the lenses in blue, the stomach in yellow, the swim bladder in red, and orbital space in purple.



organs. This could possibly cause organ damage by physically compressing or distorting organs. In the dissection of fish NVT, no displacement of internal organs was observed, despite a low to moderately overinflated swim bladder.

During rapid decompression, expanding gas escaping from the swim bladder likely ruptures the peritoneum and fills the orbital regions, forcing pressure to increase behind the eye and pushing the eye out of its socket. This leads to stretching of the optic nerve and muscles that attach the eye to the brain and skull. Additionally, despite lack of visible external trauma in fish NVT, the MRI images show a small orbital gas-filled space behind the right eye, suggesting that some tissue damage may occur even in fish without visible exophthalmia. However, these smaller pockets of gas that do not result in exophthalmia are less likely to create optic nerve stretching. In dissections, this orbital space could not be seen. Previous research on guinea pigs (*Cavia porcellus*) induced axonal injury to optic nerves by physically pulling the orbital globe out of the eye socket and stretching the optic nerves and then recording abnormalities in optic nerve cell structure (Maxwell et al. 2003). The highest numbers of damaged axons were found half way along the length of the optic nerve, depicted by dissociation of myelin lamellae and other morphological alterations (Maxwell et al. 2003). Therefore, stretching of the optic nerve in rockfish could lead to nerve damage and possible vision loss.

High-resolution T_2-weighted MR images of rockfish allowed accurate quantitative assessment of gas-filled spaces in the body, as well as displacement of the swim bladder, stomach, and eyes. Although large organs and spaces were easily segmented, smaller or very complex organ areas such as the lenses, eyes, and whole body were difficult to segment because of the limitations in scanner resolution. Much higher spatial resolution is attainable with higher field MRI scanners and will be important in performing a similar analysis on smaller structures such as the optic nerve, retina, and choroids. Swim bladder overinflation, stomach eversion, and optic nerve stretching were confirmed using dissections, but the orbital space was not visible in dissections. Segmenting body regions in rockfish can prove useful for analyzing

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morphological damage or inflation of organs and regions in rockfish specimens following barotrauma and assessing the level of damage quantitatively, without disrupting the structural integrity of gas-filled spaces or the fish as a whole. Furthermore, our study provides pertinent information on individuals of economically important groundfish populations and provides a unique method for making barotrauma-associated comparisons across all rockfish species.

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Stream fish occurrence in response to impervious cover, historic land use, and hydrogeomorphic factors

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Abstract: We evaluated competing models explaining the occurrence of five stream fishes in an urbanizing watershed to determine the relative importance of (*a*) impervious surface and other indicators of current land use, (*b*) historic land use (e.g., agriculture, impoundments), and (*c*) hydrogeomorphic characteristics (e.g., stream size, elevation, geology). For four of five species, the best-supported models were those that included both current effective impervious cover and historic land use predictor variables, although models with only effective impervious cover were equally well supported for two of those species. For the best-supported models for three species, occurrence probability was predicted to approach zero at levels of development equivalent to about 2%–4% effective impervious cover in the surrounding region. Data were drawn from 357 fish collections made in the Etowah River basin, Georgia, USA, between 1998 and 2003 and analyzed using hierarchical logistic regression accounting for imperfect species detection. This is the first study we know of to examine the response of individual fish species to both increasing impervious cover and historic land use. Such individual species assessments will be increasingly necessary to guide policies for managing urban effects and preventing extirpations of sensitive species.

Résumé : Nous évaluons plusieurs modèles concurrents explicatifs de la présence de cinq poissons d'eau courante dans un bassin versant urbanisé afin de déterminer l'importance relative (*a*) de la surface imperméable et des autres indicateurs de l'utilisation actuelle des terres, (*b*) de l'utilisation des terres dans le passé (par ex., agriculture, barrages) et (*c*) des caractéristiques hydrogéomorphiques (par ex., taille des cours d'eau, altitude, géologie). Pour quatre des cinq espèces, les modèles les plus solides sont ceux qui incluent à la fois la couverture imperméable effective actuelle et les variables prédictives de l'utilisation des terres du passé; par ailleurs, les modèles qui tiennent compte seulement de la couverture imperméable efficace fonctionnent aussi solidement avec deux de ces espèces. Dans le cas du modèle le plus solide pour trois espèces, il prédit que la probabilité d'occurrence s'approche de zéro à des niveaux de développement équivalents à environ une couverture imperméable effective de 2 % – 4 % dans la région avoisinante. Les données proviennent de 357 récoltes de poissons faites dans le bassin de l'Etowah, Géorgie, É.-U., entre 1998 et 2003 et elles ont été analysées à l'aide d'une régression logistique hiérarchique qui tient compte de la détection imparfaite des espèces. Il s'agit de la première étude, à notre connaissance, qui examine les réactions d'espèces individuelles de poissons à la fois à la croissance de la couverture imperméable et à l'utilisation des terres dans le passé. De telles évaluations d'espèces individuelles deviendront de plus en plus nécessaires afin de guider les politiques de la gestion des effets de l'urbanisation et de prévenir l'extirpation des espèces sensibles.

[Traduit par la Rédaction]

Introduction

Many studies have demonstrated that fish assemblages respond to a gradient of urbanization (e.g., Wang et al. 2001; Meador et al. 2005; Roy et al. 2005). Most such studies have used characteristics of the fish assemblage — such as an index of biotic integrity, species diversity, or a ratio of homogenization — as response variables (but see Olden 2003 and Walsh et al. 2004*b*, the latter of which is for an amphipod). The limitation of assemblage-level analyses is that they do not provide information about the response of individual species, especially rare ones. Species-level responses can be a matter of significant interest in the management of imperiled fish species. As urban land cover

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increases globally, a growing number of species will be impacted by urbanization, and knowledge of species-specific relationships between indicators of urban cover and fish occurrence or abundance will be essential to develop effective conservation strategies.

In the absence of sufficient long-term data sets relating changes in fish occurrence to land use, most studies employ a space-for-time substitution. That is, variation in species occurrence over space is related to variation in land use patterns at a fixed point in time. The space-for-time substitution requires the assumption that observed species distribution patterns are due to the effects of contemporary land use patterns. However, this assumption may not be supported. Contemporary fish distributions are likely the result of (1) complex interactions between hydrogeomorphic characteristics of streams, the landscape, and other biota (Allan 2004), and (2) past human land use activities (Harding et al. 1998), and the two are often strongly related. For example, cities tend to be built on low-slope, formerly agricultural land, rather than high-slope, formerly forested land. If we fail to account for the influence of hydrogeomorphic influences and historic land use, we risk misinterpreting the role of current land use.

This study involves fish species of the Etowah River basin, Georgia, USA. The Etowah River is a major tributary of the Coosa River system in the Mobile River basin (Fig. 1). The Etowah basin supports a diverse aquatic fauna, with 76 extant native species of fish (Burkhead et al. 1997), including three that are listed under the Endangered Species Act and six others that are considered imperiled but are not currently listed. A significant threat facing these organisms is rapid urbanization from the metropolitan Atlanta region (Wenger 2006). To head off a potential conflict between development and species protection, in 2003 the local governments of the Etowah basin began a process to develop the Etowah Aquatic Habitat Conservation Plan (HCP; Etowah HCP Advisory Committee 2006). The purpose of the plan is to implement a set of growth management policies and ordinances that minimize the impact of future development on the aquatic fauna, thus permitting additional growth without threatening the persistence of federally protected organisms.

This study was designed to evaluate whether there was sufficient evidence of an urban effect on listed fish species to justify controls on stormwater runoff as part of the HCP. Although the occurrence patterns of many Etowah fish species appear to suggest a negative relationship with urbanization, these patterns may also reflect natural landscape characteristics or past land use activities. Historic land use appears especially likely to have influenced distributions because much of the Etowah basin experienced intense row-crop agriculture from the 1800s to the early 1900s. The agricultural practices caused massive erosion and the sedimentation of stream valleys (Trimble 1974), which could have led to extirpation of sensitive fish species from many tributaries. Subsequently, many impoundments were constructed across the basin, which may have prevented recolonization and otherwise influenced (and may continue to influence) fish distributions.

In this study we examine five species native to the Etowah system (Table 1) whose distribution patterns suggest possible negative correlations to urban cover. Two of the species (*Etheostoma etowahae* and *Etheostoma scotti*) are listed under the Endangered Species Act and are targets of the Etowah HCP. Our objective is to determine the relative importance of hydrogeomorphic characteristics, historic land use, and current urbanization in explaining the distribution of the species. Our focal metric of current land use is effective impervious area (EIA), which other studies have found to be a key indicator of urban effects on aquatic biota (Walsh et al. 2005*a*). A secondary objective is to identify response thresholds of fish occurrence to increasing EIA.

Materials and methods

Data preparation

Fish collections

We selected 357 records of fish collections from a database maintained by the Georgia Museum of Natural History (Athens, Georgia). We used collections made in the Etowah basin between 1 January 1999 and 31 December 2003, which we considered approximately contemporaneous with the available "current" land cover data (see below). We selected only collections intended to characterize the full assemblage of sampled habitats using electroshocking, kick-seining, and seine hauling. Some of the data were used in previously published studies (Walters et al. 2003*a*; Roy et al. 2005). We excluded collections from streams draining less than 0.5 km^2 and those of uncertain reliability, which included collections targeting only certain species, collections that appeared to be missing information, and collections where notes indicated that an incomplete or low-effort sample had been taken. Sample reaches at sites were 50 m to 200 m in length. Collections from localities that were very close together (less than 0.5 km apart within the same stream, without large intervening tributaries) were assumed to be from the same site. However, collections from the same locality but more than 2 years apart were treated as if they were from independent sites with regard to estimating detection probability, under the assumption that populations could not be considered "closed" across this time period (see below). With these adjustments, the primary data set included 252 distinct sites, each sampled from one to five times.

We selected an additional set of 65 records for collections made at 31 sites to provide supplementary data for estimating species-specific probability of detection (following MacKenzie et al. 2002). We used sites where collections were made twice within 2 consecutive years (28 sites) or three times in 3 years (3 sites; temporal replicates) between 1 January 1990 and 31 December 1998; we also selected pairs of sites that were immediately adjacent and were sampled within a day of one another (spatial replicates) within this time period. We assumed that a species was either present or absent during samplings for each set of replicates, i.e., that the populations were closed.

Hydrogeomorphic predictor variables

For each collection site, we delineated the watershed that drained to the site and assigned it to one of 21 tributary systems (Fig. 1). We derived seven hydrogeomorphic predictor variables: watershed area, downstream link magnitude

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Fig. 1. The Etowah River basin (Georgia, USA), showing collection sites and tributary system boundaries.

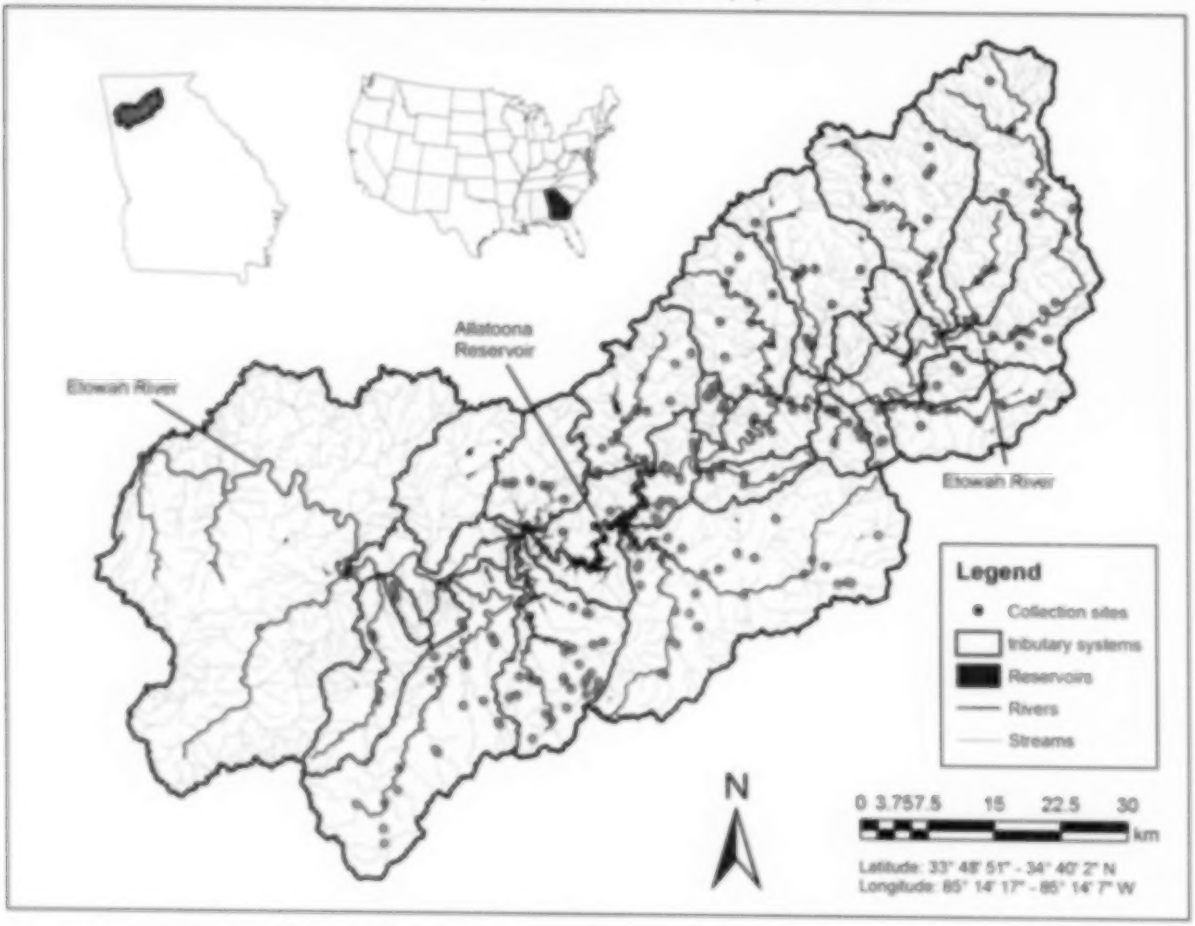

Table 1. Species analyzed for occurrence in relation to hydrogeomorphic variables, historic land use, and current land use.

Species	Family	Distribution	Status
Cyprinella trichroistia Jordan and Gilbert, tricolor shiner	Cyprinidae	Mobile River basin, AL, GA, TN	CS; sensitive to increasing turbidity (Burkhead and Jelks 2001); representative of other sensitive minnows
Noturus leptacanthus Jordan, speckled madtom	Ictaluridae	Atlantic and Gulf Slope drainages, SC to LA	CS; potential surrogate for *N.* sp. cf. *munitus*, coosa madtom (T)
Etheostoma etowahae Wood and Mayden, Etowah darter	Percidae	Etowah River system, GA	E; federally listed
Etheostoma scotti Bauer, Etnier and Burkhead, Cherokee darter	Percidae	Etowah River system, GA	T; federally listed
Percina palmaris Bailey, bronze darter	Percidae	Coosa and Tallapoosa river systems, AL, GA	CS; potential surrogate for *P. antesella* Williams and Etnier, amber darter (E)

Note: Status follows Warren et al. 2000: currently stable (CS), threatened (T), and endangered (E).

(d-link), elevation, physiographic province, bedrock geology, surficial geology, and stream slope (Table 2). All were calculated in ArcView 3.3 or ArcGIS 9.0 software (ESRI, Redlands, California). Watershed area was calculated as the total area draining to the collection site and served as an indicator of stream size at the fish sampling location. D-link was used as a way of describing a stream reach's position in a watershed — whether it was a headwater stream or directly connected to larger main-stem streams, for example. D-link was calculated as the number of unbranched streams draining to the next confluence downstream of the site, using 1:24 000 scale maps (Osborne and Wiley 1992). Elevation was calculated at the collection site from 30 m resolution digital elevation models (DEMs) (US Geological Survey 1988). Physiographic province (Georgia Geologic Survey 1999), bedrock geology summarized by group

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Table 2. Summary statistics on continuous predictor variables measured for 252 collection sites used in models of species occurrence.

Variable	Abbrev.	Group	Mean	SD	Min.	Max.
Watershed area (km^2)	area	Hydrogeo	9.3	28.3	0.5	1591
Elevation (m above sea level)	elev	Hydrogeo	305.01	53.06	207.43	536.52
Downstream link magnitude	d-link	Hydrogeo	56.5	118.4	0.00	415
Density of dams in watershed (no./km^{-2})	damw	Historic	0.98	0.87	0.00	5.95
% of watershed in impoundments	waterw	Historic	0.70	0.64	0.00	3.73
% watershed in intense historic land use	histw	Historic	33.39	22.48	0.00	90.69
% watershed urban cover	urbanw	Current	16.11	17.60	0.00	73.12
% watershed forest cover	forestw	Current	65.12	17.74	0.00	99.15
% watershed TIA	tiaw	Current	3.83	5.55	0.01	28.37
% TIA in 500 m radius	tia500	Current	3.32	5.39	0.00	29.78
% TIA in 1 km radius	tia1	Current	3.47	5.41	0.00	28.89
% TIA in 1.5 km radius	tia15	Current	3.57	5.23	0.00	26.37
% TIA in 2 km radius	tia2	Current	3.71	5.26	0.02	24.71
% watershed EIA	eiaw	Current	2.49	4.44	0.00	23.67
% EIA in 500 m radius	eia500	Current	2.07	4.33	0.00	26.23
% EIA in 1 km radius	eia1	Current	2.23	4.34	0.00	24.56
% EIA in 1.5 km radius	eia1.5	Current	2.32	4.14	0.00	22.07
% EIA in 2 km radius	eia2	Current	2.44	4.19	0.00	20.41
Mean slope of large streams in tributary system (Δ elev./watershed area)	slope	Hydrogeo	83.75	73.03	14.62	289.64
Density of dams in tributary system (no./km^{-2})	damswden	Hydrogeo	0.83	0.41	0.29	1.90
% of tributary system in impoundments	watertr	Hydrogeo	0.58	0.37	0.12	1.35
% tributary system in intense historic land use	histtr	Hydrogeo	32.37	18.60	4.84	61.47
Presence of metamorphic mafic bedrock geology group (binary)	meta. mafic	Hydrogeo	—	—	—	—
Clayey sand / sandy clay quaternary geology (binary)	rck	Hydrogeo	—	—	—	—
Micaceous saprolite quaternary geology (binary)	sob	Hydrogeo	—	—	—	—
Multiple binary bedrock geology groups	geology	Hydrogeo	—	—	—	—

Note: TIA, total impervious area; EIA, effective impervious area. "Abbrev." indicates the abbreviation used for the variable in other tables. "Group" refers to whether the variable is classed as hydrogeomorphic ("Hydrogeo"), historic land use, or current land use. SD, standard deviation; "Min." and "Max." indicate the minimum and maximum values, respectively.

(Georgia Geologic Survey 1999), and surficial (quaternary) geology (Richmond et al. 1987) were included as candidate measures of the influence of geology on physicochemical properties of streams. Local stream slope data were not available for most collection sites, so we estimated the mean slope of all streams draining at least 10 km^2 at the scale of each system using 30 m DEMs (US Geological Survey 1988), and assigned the mean to each site in that system.

Impervious area and other measures of current land cover

We focused on impervious area as an indicator of urbanization, as stormwater runoff from impervious surfaces has been identified as the primary source of stressors to urban streams (Walsh et al. 2005*b*). Previous researchers have suggested that the most problematic impervious surfaces are those that are directly connected to streams via drainage and conveyance systems (Alley and Veenhuis 1983; Booth and Jackson 1997; Walsh et al. 2005*a*). Studies have demonstrated that this effective impervious area (EIA) is a better predictor of stream biological and chemical response than total impervious area (TIA) (e.g., Wang et al. 2001; Hatt et al. 2004; Walsh et al. 2004*a*). The 2001 National Land Cover Database Zone 54 Imperviousness Layer (US Geological Survey 2003) was used as the source for TIA. This is a raster coverage with a resolution of 30 m derived from supervised classification of LandSat satellite imagery. To calculate EIA, we followed Alley and Veenhuis (1983) in developing our own empirical relationship between TIA and EIA, which we applied to the TIA coverage. We hand-delineated both impervious and directly connected impervious surfaces (which we considered EIA) from high-resolution aerial photographs for 15 sites of 25–70 ha in size. Impervious areas included roofs, roads, parking lots, sidewalks, and any other artificial impervious surfaces distinguishable on the aerial photos. Directly connected impervious surfaces were a subset of impervious surfaces that were visually noted to drain to the stormwater conveyance network. Such connections were generally obvious from the high-resolution aerial photographs, although there was occasional ambiguity; uncertain cases were considered directly connected. We then determined the relationship between TIA and EIA by fitting the data to different candidate models. The best model, selected on the basis of the coefficient of determination, was linear with a threshold:

$$(1) \qquad \text{EIA} = (1.046 \times \text{TIA}) - 6.23\%$$

where EIA = 0 for TIA values less than 6.23% ($R^2 = 0.98$).

We applied this formula cell-by-cell to the TIA layer to create a raster EIA layer. For each fish collection site, we then calculated TIA and EIA at five scales: impervious area in the watershed upstream of the site, and impervious area

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within 0.5 km, 1 km, 1.5 km, and 2 km radiuses of the site (Table 2). Note that summarizing these values across many cells reduced the differences between EIA and TIA below that expected from the above relationship. The reason was that (especially at low development levels) impervious cover was not distributed evenly across the landscape but tended to occur in patches of cells of moderate TIA surrounded by cells of zero TIA. The transformation calculation of TIA to EIA was applied only to cells with TIA values greater than zero. Therefore, in practice, a watershed of 4% TIA equated to about 2.0%–2.5% EIA. In addition to impervious cover, we also considered urban land cover and forested land cover as indicators of current land use. These variables were calculated for the upstream watershed for each site using 2001 land cover data (Kramer 2004) (Table 2).

Historic land cover

We investigated three candidate indicators of historic land cover, each measured at two scales. The first was historic modified land cover in the basin, which was quantified from 1938 aerial photographs. These were the oldest aerial photographs available for the entire region, and the best representation we could find of land use from the era of cotton production. We georectified scans of 1938 Agricultural Stabilization and Conservation Service (ASCS) 1:100 000 scale aerial photograph index sheets from the Georgia Aerial Photographs database (http://dbs.galib.uga.edu/gaph/html/). We classified the resulting images into forested areas and agricultural or developed land based on cell brightness.

The other candidate indicators of historic land use were the number and area of reservoirs, which we expected to correlate with historic land cover. Many reservoirs were built in the 1950s through the 1970s on agricultural lands, and these can be viewed both as indicators of agricultural influences and as potential stressors. All indicators of historic land use (percentage of drainage area in agricultural land cover in 1938, number of reservoirs, and area of reservoirs) were measured at two scales: (1) the watershed above each collection site and (2) the tributary system within which the collection site was nested (Table 2).

Data analysis

One of our goals in the modeling was to obtain covariate parameter estimates with minimal bias by accounting for spatial dependencies in the data and incomplete detectability of species. Failure to account for spatial correlations can lead to underestimates of the variance of parameter estimates (Snijders and Bosker 1999), while failure to correct for incomplete detection can lead to bias in the means of parameter estimates (Gu and Swihart 2004). We constructed logistic regression models using two-level hierarchical modeling to manage spatial correlations (Snijders and Bosker 1999), following the general approach for modeling species distributions outlined by Latimer et al. (2006). We adapted this to account for incomplete detectability using a species occupancy model (MacKenzie et al. 2002). While incorporating both hierarchy and incomplete detectability into a logistic regression model presents significant challenges for conventional maximum likelihood estimation, Monte Carlo Markov Chain (MCMC) model-fitting techniques are able to accommodate such complexity (Conroy et al. 2005; Peterson et al. 2005). The disadvantage of MCMC techniques is that model fitting is computer-intensive and time-consuming. Therefore, we used a two-stage modeling approach: (1) We screened potential predictor variables representing hydrogeomorphic characteristics, historic land use, and current land use with ordinary logistic regression that assumed complete detectability. (2) We evaluated the relative fit of the best-supported models from the initial screening using hierarchical species occupancy models.

Screening of candidate predictor variables

For the candidate predictor variable selection, we ignored spatial dependencies and assumed complete detectability. For sites with multiple collections, we assumed a species was present if it was encountered in any of the collections. To increase linearity in the predictor variables, watershed area was square root transformed and d-link was natural log transformed. All continuous predictors were normalized with a mean of 0 and standard deviation of 1 and we included quadratic terms for area, d-link, and elevation as possible predictors.

We evaluated a series of logistic regression models for each of the five species, with species occurrence as the dependent variable. All models were run with the statistical package R 2.0.1 (R Development Core Team 2004). There were four model categories: (1) models with only hydrogeomorphic predictors, or "hydrogeomorphic", (2) models with hydrogeomorphic predictors plus a historic land use predictor, or "historic", (3) models with hydrogeomorphic predictors plus a current land use predictor, or "current", and (4) models with hydrogeomorphic predictors plus a historic and a current land use predictor, or "global". Our goal was to identify the best-approximating model in each category (1 through 4) for each species, using Akaike's information criterion modified for small sample size (AIC_c) as the basis for selection (Burnham and Anderson 2002). To identify the most plausible model that included only hydrogeomorphic predictor variables, we fitted a model with all hydrogeomorphic predictors and then performed a series of stepwise removals, selecting the three best-supported models based on AIC_c. Next, we compared 18 models, each of which was based on one of the three best-supported hydrogeomorphic predictor models and included one of the six candidate historic land cover predictor variables. We again performed a stepwise removal of variables to determine whether a reduced model was better supported. We then repeated the process for the 12 candidate predictor variables for current land use (comparing 36 models). Finally, we compared models with different combinations of both a historic land use predictor variable and a current land use predictor variable, to select the best global model. We compared the best-supported models in each category with one another to estimate which was best supported overall.

Hierarchical occupancy modeling

To account for incomplete detection, we jointly modeled species detectability and presence as

$$(2) \qquad P(\mathrm{d}) = P(\mathrm{d}|\mathrm{c})P(\mathrm{c})$$

where $P(\mathrm{d})$ is the probability that the species is present and detected at the site, $P(\mathrm{c})$ is the probability that the species

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is present, and $P(\text{dl}|\psi)$ is the probability that the species is detected given that it is present, i.e., detectability. Detection probability was calculated using a site occupancy model (MacKenzie et al. 2002) based on the encounter history of the species at sites sampled multiple times. Briefly, this method simultaneously estimates species presence and detectability by assuming that the occupancy state (presence or absence) of a site sampled multiple times does not change between samples; thus, if a species is found three out of four times when a site is sampled in rapid succession, its detectability is 75%. In our data set, 63 sites were sampled a second time, 21 of those were sampled a third time, 13 of those were sampled a fourth time, and 8 of those were sampled a fifth time (the remaining 189 sites were sampled only once each). The assumption that populations remain unchanged ("closed") between samples was likely violated in many cases, which adds slightly to the uncertainty of the detectability estimates. To improve our estimate of detectability, we added a second data set of an additional 31 sites sampled at least twice during the period 1990–1998. We assumed that because collection methods remained relatively consistent through the 1990s and early 2000s, species detectability was also consistent. The MCMC methods we employed permitted us to construct models in which these auxiliary data informed the estimate of $P(\text{dl}|\psi)$ but not any of the other model parameters. That is, the detectability parameter for each species was estimated across both data sets, but parameters for explaining presence–absence were estimated using only the main data set. It is possible to include covariates of detectability to account for differences in sample effort and methods, but we did not do so because we lacked relevant collection-level data across all samples.

Prior to running the hierarchical occupancy models, we tested for spatial autocorrelation at the level of the tributary systems by performing an analysis of variance on the residuals of each of the best-supported screening models, using the tributary systems as treatments. We detected significant dependence within the tributary systems ($P < 0.001$), indicating that samples within the same tributary system tended to be similar to one another and could not be considered fully independent samples. If ignored, this problem would tend to produce overly narrow credible intervals for model parameters (Snijders and Bosker 1999). We addressed the issue by defining a two-level hierarchical structure with sites nested within the 21 tributary systems, implemented in the model by adding a normally distributed random effect at level two (Snijders and Bosker 1999). Level one of the model can be represented as

$$\text{(3)} \qquad \text{logit}(P(\psi_{i,j})) = \beta_0 + \sum_{s=1}^{m} \beta_s x_{s,i,j}$$

where $x_{s,i,j}$ are $s = 1, 2, \ldots m$ predictors for site i within tributary system j. The intercept is then modeled as a function of tributary system characteristics (level two):

$$\text{(4)} \qquad \beta_0 = \gamma_0 + \sum_{r=1}^{n} \gamma_r w_{r,j} + \delta_j$$

where $w_{r,j}$ are the $r = 1, 2, \ldots n$ predictors corresponding to tributary system j, and δ_j is a random effect that varies normally among reaches with a mean of zero and variance σ_j^2.

For each of the five species, we fit the best-supported screening model in each of the four categories (hydrogeomorphic, historic, current, and global) to the hierarchical occupancy models. If the screening analyses showed that the second- or third-best models in a category also had considerable support, we also fit these to the hierarchical occupancy models. We used MCMC methods as implemented in WinBUGS 1.4 (Spiegelhalter et al. 2003) for all hierarchical occupancy modeling. We ran six parallel chains and tested each model for convergence using the Gelman–Rubin diagnostic (Gelman and Rubin 1992). Models converged within 8000 iterations, and the values from this "burn in" period were discarded. Models were then run for a further 60 000 iterations to estimate parameters and deviance. We used diffuse priors for all parameters. To reduce MCMC autocorrelation, models were thinned by a factor of 10, which means that only every tenth sample was used in calculating statistics. The use of this technique greatly reduced autocorrelation but did not eliminate it in all cases. We tested increased iterations with even greater thinning, up to 600 000 iterations with 100× thinning, but parameter estimates, deviance, and convergence diagnostics remained stable throughout the range of iterations evaluated. Therefore, we considered 10× thinning adequate.

We used threefold cross-validation to select models and estimate their out-of-sample predictive performance. For test sites we assumed that tributary system membership was unknown. We ranked models by their predictive performance using the area under the curve (AUC) of the receiver-operating characteristic (ROC) plot as a summary statistic. The ROC curve is the ratio of true positives to false positives when the species occurrence decision threshold is varied between zero and one; the AUC of the ROC curve is considered a robust measure that is invariant to species prevalence (Manel et al. 2001; Olden et al. 2002; Latimer et al. 2006).

We found that for models with large variances on the random effect, fixed-effect parameter estimates were proportionately large. These large values are a result of the fixed level one variance of logistic regression models, which leads to inflation of parameter estimates to maintain proportionality between levels one (e.g., stream site) and two (e.g., tributary) when random effects are added (Snijders and Bosker 1999). We corrected for this phenomenon by standardizing the parameter estimates of each model by the sum of level one and level two variances. These standardized values were used to calculate odds ratios for the mean and 90% credible intervals for the fixed-effect parameter estimates for all variables of the best-supported current, historic, and global models.

Results

Impervious cover, historic land cover, and reservoir density varied considerably across the basin and among tributary systems (Figs. 2 and 3), resulting in high variances of most variables across the collection sites or watersheds (Table 2). As expected, there were correlations between historic land use predictor variables and current land use pre-

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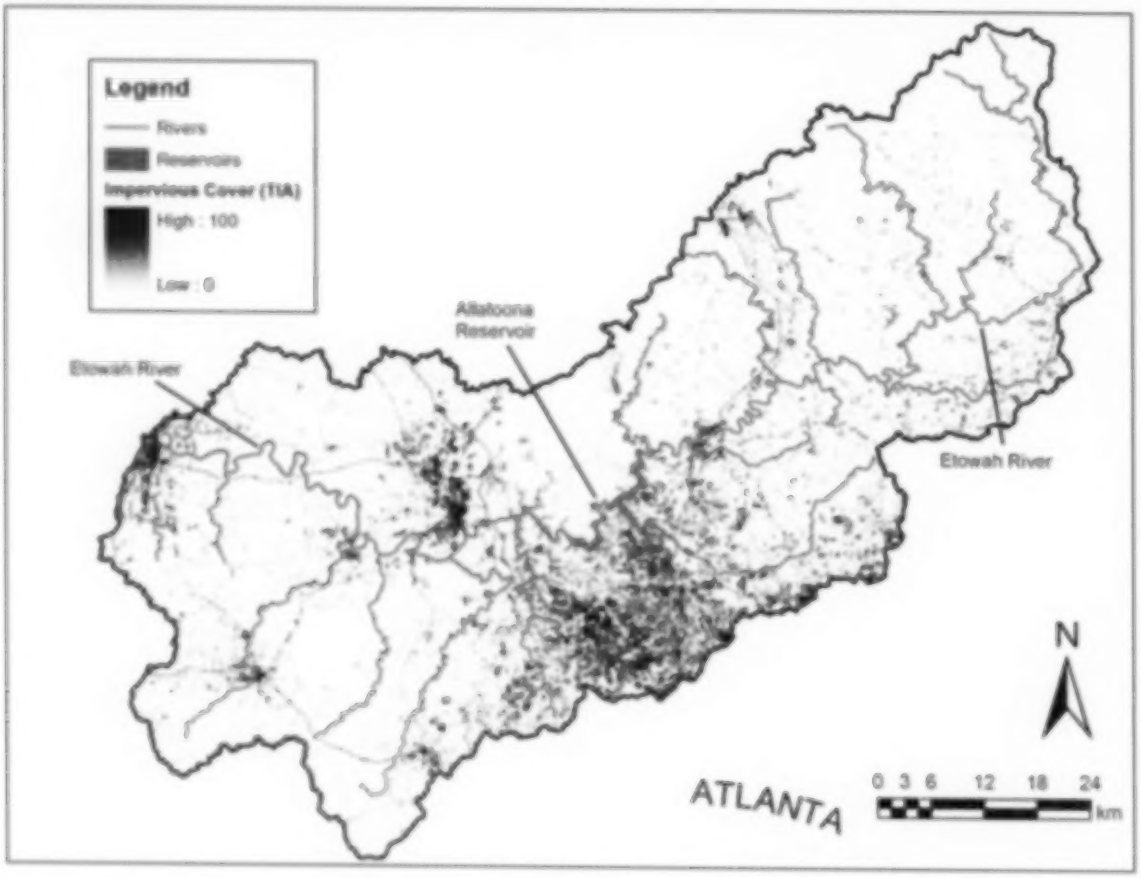
Fig. 2. Total impervious area in the Etowah basin.



dictor variables, but pairwise Pearson correlations between historic and current land use predictors were less than 0.6 in all cases and we observed no evidence that multicollinearity affected parameter estimates.

Based on the screening model analysis, we selected 7–14 models to run for each species in the hierarchical occupancy modeling (Table 3). Among the hydrogeomorphic predictor variables evaluated, watershed area, downstream link magnitude, tributary system slope, and elevation were the most commonly included in the best-fitting models. Among historic land use predictors, area inundated by impoundments was the most commonly included, but historic land cover was included for at least some models for two species. Effective impervious area (EIA) within 500 m to 1.5 km of the collection site was the most common measure of current land use selected for inclusion in models, based on the screening analysis. The only exception was *E. scotti*, for which forest cover in the watershed was the best current land use predictor.

Gelman–Rubin convergence diagnostics showed that all of the hierarchical occupancy models converged. Based on the AUC values, the best model for each species was a global model, with the exception of *E. scotti*, for which a historic model (i.e., one without a predictor variable for current land use) was best supported (Table 3). However, for *N. leptacanthus* and *E. etowahae* the best current land use model (i.e., one without a predictor variable for historic land use) was equally or nearly equally well supported. The best historic land use model was less well supported than the best current land use model for all species except *E. scotti*. For all species, the best hydrogeomorphic model was a poorer predictor than the best global, historic, and current models. The differences among the AUC scores for the best models for each species were small. According to a rule of thumb (Swets 1988), models with AUC values > 0.9 have high accuracy. The best models for three of the species met this threshold, while those for *N. leptacanthus* had slightly lower AUC values and those for *E. scotti* had substantially lower AUC values (Table 3).

Cross-validation inherently penalizes model overfitting, and this was evidenced in our analyses by the higher AUC scores of some models that were subsets of others. For example, *N. leptacanthus* model 8 was a subset of models 1–7, but was ranked equal to or higher than these more complex models, indicating that it was the more parsimonious model. Nevertheless, some highly ranked models included parameters whose credible intervals overlapped 1 (Table 4), indicating uncertainty in whether the direction of

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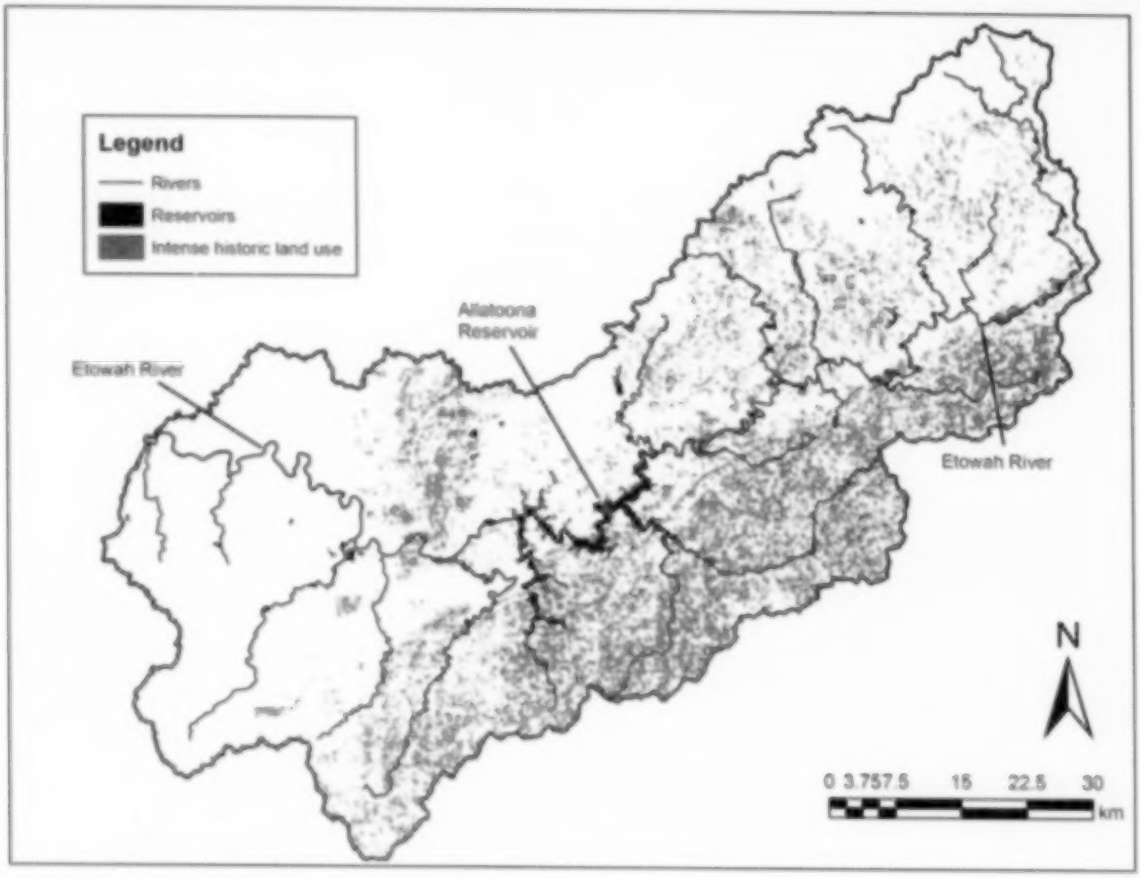
Fig. 3. Historic modified land cover in the Etowah basin.



the correlation was positive (values greater than 1) or negative (values less than 1). For example, for *N. leptacanthus* model 5, the credible interval for historic land use ranged from 0.08 (a strong negative effect) to 1.80 (a moderate positive effect). The historic land use term was therefore of questionable predictive value, and indeed the simpler model 8, which lacked this parameter, had an AUC score equal to that of model 5.

The species with the strongest relationship with current land use was *C. trichroistia* (Table 4). Using the best predicting model, we estimated that the species was almost 20 times less likely to occur for each 1% increase in EIA within 1.5 km (note that this was not a relative increase in EIA, but an absolute increase; e.g., a change from 5% to 6% would be a 1% increase). Occurrence probability approached zero when EIA exceeded about 2% and other predictor variables were held to their mean values (Fig. 4). The presence of *P. palmaris* and the presence of *E. etowahae* also were strongly negatively related to EIA, although the 90% credible interval for *E. etowahae* was very broad (Table 4). For both species the models predicted that the occurrence probabilities approached zero at 4% EIA and above, using the mean estimates for the EIA parameter (Fig. 4). *Noturus leptacanthus* showed a weaker relationship with EIA, and *E. scotti* showed essentially no relationship, with the mean credible interval for the odds ratio centered near 1 and broadly overlapping on either side (Table 4, Fig. 4).

Under the highest-ranked models, the distributions of three species showed a strong relationship with historic land use (Table 4). Using the mean parameter estimates of the best-supported models for *C. trichroistia*, *E. etowahae*, and *P. palmaris*, species were 1.7 to 2.5 times less likely to occur for each increase of 0.25% in the area of upstream watershed or tributary system that was impounded. *Noturus leptacanthus* was 2.3 times less likely to occur for each 10% increase in the area of the upstream watershed in historic modified land cover, but the credible interval of the odds ratio was very wide and included 1, indicating large uncertainty around the species' response. The odds ratio credible interval for *E. scotti* was centered near 1, suggesting little relationship with historic land use.

The current distribution of four of the five species was positively related to watershed area and downstream link magnitude, usually with a second-order term indicating lower occurrence probability in the largest streams. The exception was *E. scotti* occurrence, which was negatively related to watershed area (Table 4). The best-supported model

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Table 3. Hierarchical occupancy models for each species with model selection statistics.

Model No.	Category	Predictor variables	AUC
Cyprinella trichroistia			
2	**Global**	**area, area^2, d-link, d-link^2, slope, waterw, cia1.5**	**0.933**
1	Global	area, area^2, d-link, d-link^2, waterw, cia1.5	0.931
4	Global	area, area^2, d-link, d-link^2, histtr, cia1.5	0.929
3	Global	area, area^2, d-link, d-link^2, slope, histtr, cia1.5	0.926
5	**Current**	**area, area^2, d-link, d-link^2, cia1.5**	**0.922**
6	Current	area, area^2, d-link, d-link^2, slope, cia1.5	0.922
8	**Historic**	**area, area^2, d-link, d-link^2, histtr**	**0.915**
7	Historic	area, area^2, d-link, d-link^2, slope, histtr	0.904
9	Historic	area, area^2, d-link, d-link^2, waterw	0.875
10	**Hydrogeomorphic**	**area, area^2, d-link, d-link^2, slope**	**0.788**
Noturus leptacanthus			
5	**Global**	**area, slope, histw, cia1**	**0.892**
8	**Current**	**area, slope, cia1**	**0.892**
7	Current	area, elev, slope, cia1	0.887
4	Global	area, d-link^2, elev^2, slope, histw, cia1	0.885
6	Current	area, d-link^2, elev^2, slope, cia1	0.878
3	Global	area, elev, slope, histtr, cia1	0.875
2	Global	area, slope, histtr, cia1	0.874
1	Global	area, d-link^2, elev^2, slope, histtr, cia1	0.873
12	**Historic**	**area, slope, histtr**	**0.858**
14	**Hydrogeomorphic**	**area, slope**	**0.855**
11	Historic	area, elev, slope, histtr	0.851
9	Historic	area, d-link^2, elev^2, slope, histw	0.843
10	Historic	area, d-link^2, elev^2, slope, histtr	0.834
13	Hydrogeomorphic	area, d-link^2, elev^2, slope	0.814
Etheostoma cinereum			
3	**Global**	**area, d-link, d-link^2, slope, watertr, cia1.5**	**0.946**
1	**Current**	**area, d-link, d-link^2, slope, cia1.5**	**0.945**
6	Global	area, d-link, d-link^2, slope, damswden, cia1.5	0.945
4	Global	area, area^2, d-link, d-link^2, slope, watertr, cia1.5	0.943
7	**Historic**	**area, area^2, d-link, d-link^2, slope, damswden**	**0.936**
5	Global	area, d-link, d-link^2, watertr, cia1.5	0.932
9	Historic	area, area^2, d-link, d-link^2, watertr, slope	0.931
2	Current	area, d-link, d-link^2, cia1.5	0.927
11	**Hydrogeomorphic**	**area, area^2, d-link, d-link^2, slope**	**0.912**
8	Historic	area, d-link, d-link^2, damswden	0.903
10	Hydrogeomorphic	area, area^2, d-link, d-link^2, elev, elev^2, slope	0.896
Etheostoma scotti			
4	**Historic**	**area, elev^2, meta. mafic, rck, snb, watertr**	**0.737**
5	**Global**	**area, elev^2, meta. mafic, rck, snb, watertr, forestw**	**0.727**
2	**Current**	**area, elev^2, meta. mafic, rck, snb, forestw**	**0.724**
7	**Hydrogeomorphic**	**area, elev^2, meta. mafic, rck, snb**	**0.711**
3	Historic	area, elev, meta. mafic, rck, snb, watertr	0.700
6	Hydrogeomorphic	area, d-link^2, elev^2, geology	0.675
1	Current	area, d-link^2, elev^2, forestw	0.624
Percina palmaris			
1	**Global**	**area, area^2, d-link, d-link^2, elev, slope, watertr, cia500**	**0.921**
2	Global	area, d-link, d-link^2, elev, watertr, cia500	0.918
6	**Current**	**area, d-link, d-link^2, elev, cia500**	**0.908**
5	Current	area, area^2, d-link, d-link^2, elev, slope, cia500	0.905
3	**Historic**	**area, area^2, d-link, d-link^2, elev, slope, watertr**	**0.895**
4	Historic	area, d-link, d-link^2, elev, watertr	0.891
7	**Hydrogeomorphic**	**area, area^2, d-link, d-link^2, elev, slope**	**0.870**
8	Hydrogeomorphic	area, d-link, d-link^2, elev	0.853

Note: The best model in each category for each species is shown in bold. Models are shown sorted from best to worst fitting based on area under the curve (AUC) of the receiver-operating characteristic plot. Variable abbreviations are defined in Table 2.

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Table 4. Parameter estimates for detection probability, intercepts, and fixed effects of best-supported hierarchical occupancy models for each species.

Parameter	Estimate	5% CI	95% CI	Unit of increase
Cyprinella trichroistia				
Detection probability	82%	75%	88%	
Intercept	1%	0%	15%	
area	37.83	5.46	340.22	Standard deviation
area^2	0.48	0.23	0.86	Standard deviation
d-link	1.79	0.59	5.64	Standard deviation
d-link^2	0.22	0.04	0.69	Standard deviation
slope	1.97	0.89	4.31	Standard deviation
waterw	0.57	0.37	0.80	0.25%
eia1.5	0.05	0.01	0.29	1%
Level 2 random effect variance	3.28	0.43	8.08	
Noturus leptacanthus				
Detection probability	55%	44%	67%	
Intercept	24%	10%	46%	
area	9.14	2.85	44.41	Standard deviation
slope	0.11	0.02	0.43	Standard deviation
histw	0.44	0.08	1.80	10%
eia1	0.70	0.50	0.88	
Level 2 random effect variance	27.94	6.46	60.82	
Etheostoma etowahae				
Detection probability	55%	44%	65%	
Intercept	1%	0%	22%	
area	17.13	2.95	372.81	Standard deviation
d-link	80.02	6.39	6664.24	Standard deviation
d-link^2	0.01	0.00	0.16	Standard deviation
slope	4.36	0.93	32.95	Standard deviation
watertr	0.41	0.14	0.95	0.25%
eia1.5	0.19	0.01	0.85	1%
Level 2 random effect variance	15.62	4.64	34.64	
Etheostoma scotti				
Detection probability	81%	75%	86%	
Intercept	73%	55%	86%	
area	0.74	0.50	0.93	Standard deviation
elev^2	0.84	0.66	0.98	Standard deviation
watertr	0.95	0.53	1.67	0.25%
meta. mafic	0.48	0.19	0.88	Present (binary)
rck	121.58	0.12	4.40×10^7	Present (binary)
ssb	0.47	0.19	0.85	Present (binary)
Level 2 random effect variance	7.78	3.02	16.86	
Percina palmaris				
Detection probability	86%	79%	92%	
Intercept	6%	0%	41%	
area	47.09	5.67	782.68	Standard deviation
area^2	0.88	0.40	2.20	Standard deviation
d-link	3.23	1.27	9.79	Standard deviation
d-link^2	0.23	0.05	0.77	Standard deviation
elev	1.47	0.79	3.28	Standard deviation
slope	1.55	0.60	3.80	Standard deviation
watertr	0.44	0.23	0.85	0.25%
eia500	0.19	0.04	0.57	1%
Level 2 random effect variance	5.26	1.16	17.39	

Note: CI, credible interval. Detection probability estimates are given as percentages. For the intercept term, estimates correspond to site occupancy (occurrence probability) when other parameters are zero. For fixed effects, values are given as odds ratios per specified unit of increase. A value greater than 1 indicates a positive correlation, and a value less than 1 indicates a negative correlation. For example, *Cyprinella trichroistia* is 95% less likely to occur for each 1% increase in EIA within 1.5 km. A credible interval that overlaps 1 indicates a variable of uncertain effect. For the level 2 random effect, values are variance estimates.

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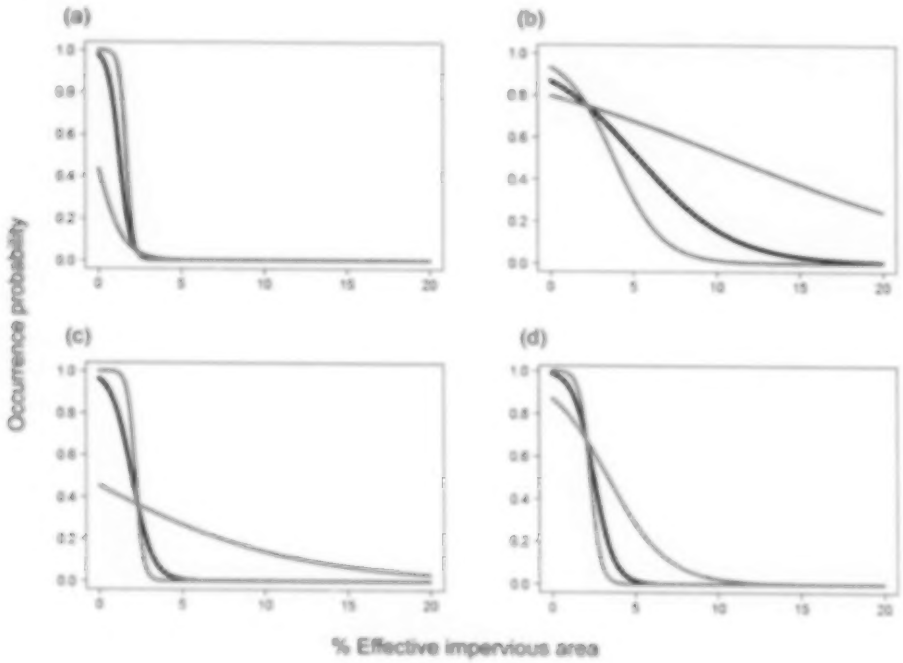
Fig. 4. Occurrence probability of each species under the best-supported model in response to increasing impervious cover. The black line represents the response curve based on the mean parameter estimate for effective impervious area (EIA); gray lines represent the response curves based on the 5% and 95% values for the parameter estimate for EIA. Predictor values for watershed area and d-link are set to one standard deviation larger than the mean, while other predictors are set to mean values. (*a*) *Cyprinella trichroistia*; (*b*) *Noturus leptacanthus*; (*c*) *Etheostoma etowahae*; (*d*) *Percina palmaris*. *Etheostoma scotti* is not plotted because the best-supported model did not include a current land use predictor variable.



for *P. palmaris* indicated that species occurrence was positively related to elevation, whereas *E. scotti* occurrence was negatively related to elevation. Slope was positively related with the occurrence of *C. trichroistia*, *E. etowahae*, and *P. palmaris*, although in all cases the 90% credible interval overlapped 1. The occurrence of *N. leptacanthus* was negatively related to slope (Table 4).

There was unexplained variation at the tributary system level, as indicated by the level 2 random effect variance estimates (Table 4). Variation was greatest for *N. leptacanthus* and lowest for *C. trichroistia*. The variability among tributary systems is reflected in shifted intercepts, which affect the relationship of fixed effects (such as EIA) by shifting the curve to the left or right of the overall mean (Fig. 5, using *E. etowahae* as an example).

Discussion

We found that for four of the five species evaluated, both historic and current land use variables were included in the best-supported hierarchical occupancy models. For *N. leptacanthus* and *E. etowahae*, the best model was a toss-up between one including current land use (and not historic land use) and one with both current and historic land use, suggesting that historic land use added relatively little explanatory power. The results provide good evidence that both current and historic land use are potentially important determinants of occupancy patterns for these species. However, the similarity in AUC scores among the best models for each species suggests that caution is warranted in determining which model is best. Although we know of no standard criteria for identifying a candidate set of well-supported models based on AUC scores, we considered it prudent to consider any model with a score within 0.010–0.015 of the top model to be an alternative hypothesis with good support from the data. By this criterion, both the current land use and global models are well-supported alternatives for *C. trichroistia*, *N. leptacanthus*, and *P. palmaris*, while the global, current, and historic models are supported for *E. etowahae* and *E. scotti*.

Based on the top models, the occurrence of several species was strongly related to low levels of EIA. Many previous studies have reported declines in aquatic fauna in watersheds draining more than 10%–12% impervious cover (Klein 1979; Schueler 1994; Wang et al. 2000). Our results indicate that some species become rare at impervious cover levels as low as 2% EIA. For some species there is considerable uncertainty around this value, but for *C. trichroistia* this threshold is consistent across all credible intervals of all well-supported models. We caution that the accuracy of these values is somewhat uncertain because the accuracy and bias of the impervious cover layer (US Geological Survey 2003) on which EIA is based are unreported in the metadata and apparently unmeasured. We anecdotally

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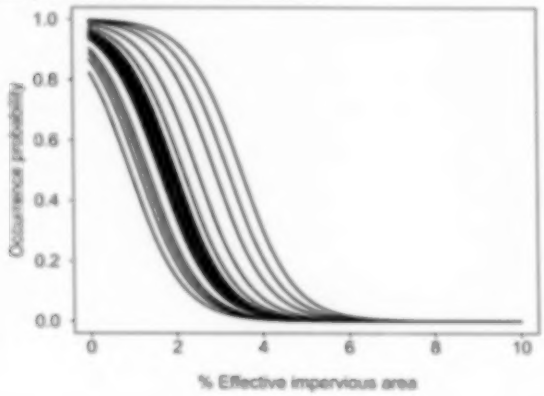
Fig. 5. Occurrence probability for *Etheostoma etowahae* in each of 21 tributary systems as a function of increasing EIA under the best-supported model. Coefficients for fixed and random effects are held to their mean estimates. Predictor values for watershed area and d-link are set to one standard deviation larger than the mean, while other predictors are set to mean values.



observed that impervious cover appeared to be underestimated in the data layer in some geographic regions; we suspect this may be a common problem with impervious layers derived from satellite imagery. Nevertheless, our observed 2% EIA threshold value for the most sensitive species is consistent with a recent study that suggested EIA must remain below 2% to maintain natural ecological conditions (Ladson et al. 2006).

We found that for four of five species, models with current land use (and not historic land use) provided slightly more accurate predictions than models with historic land use (and not current land use). Our finding is in contrast to the results of Harding et al. (1998), who found that 1950s land use was a better predictor of fish and invertebrate diversity than current land use. However, Harding et al. (1998) examined only forested and agricultural watersheds, whereas we considered urban watersheds as well. We found that models including both current and historic land use predictors were among the best supported for most species, providing evidence that current fish distributions are the product of past land use legacies and recent activities, especially urban development. We hypothesize that in the Etowah basin, historic agricultural activities caused extirpations of some species from large sections of the basin. The decline in agriculture and subsequent reforestation then allowed a slow recovery of these species, but current urbanization and suburbanization activities are now causing new extirpations. Such a pattern is probably evident in much of North America, owing to the widespread conversion of current and former agricultural land to urban and suburban uses.

Of the historic land use variables, density of and area inundated by impoundments were generally better predictors than historic modified land cover as mapped from aerial photos. Historic land cover may have performed less well because it was only a snapshot at one point in time (1938) and perhaps not an accurate predictor of the locations that suffered the greatest impacts. Cotton agriculture in Georgia crashed in the early 20th century with the arrival of the boll weevil (Haney et al. 1996), and by 1938 some agricultural areas may already have been abandoned and reforested. Reservoirs (many constructed for sediment control on agricultural lands) may be a better long-term signal of past land use because they are pervasive and because they may prevent recolonization of areas of extirpation. In this sense, reservoirs are both indicators of past land use and current stressors in their own right. In addition to blocking fish movement, reservoirs also eliminate fluvial habitat; alter flows, temperature, and chemistry; and have been shown to affect downstream fish assemblages (Collier et al. 1996; Freeman and Marcinek 2006). In our data set, the number of dams in the tributary system was highly correlated with the historic modified land cover in the tributary system (r = 0.88). However, the area inundated by impoundments in the collection watershed, which was a good predictor for some species, was relatively uncorrelated with historic modified land cover (r = 0.34).

The appearance of slope in the best-supported models for most species is consistent with the findings of previous studies in the Etowah basin, which identified slope (i.e., stream channel gradient) as a critical variable influencing the distribution of many species (Walters et al. 2003*a*, 2003*b*). The strengths of the relationships are somewhat remarkable considering that we were able to use only map slope and not field-measured reach slope, which was unavailable for many sites. We measured slope at the tributary system scale under the assumption that tributary systems with lower average slope had less riffle habitat, which made these systems less suited to riffle-dwelling species such as the five modeled here. Thus, at the tributary system scale mean slope is a potential filtering mechanism (Poff 1997), limiting whether fish species are likely to be present or absent from the system as a whole (we also hypothesized that low slope could indicate high suitability for agriculture, but we found no relationship between slope and historic land cover at the tributary system scale; r = 0.2). Reach-scale slope may serve as a second filter, limiting whether a species is locally present, given its presence in the tributary system.

Our study did not evaluate mechanisms by which urbanization affects fishes. Urbanization is a complex phenomenon that can impact fish populations through multiple potentially interacting pathways, including modification of the hydrologic regime, introduction of toxins, physical alteration of habitat, and reduction and shifts in the food base (see Paul and Meyer 2001; Walsh et al. 2005*b*; Wenger 2006). Past studies in the Etowah basin have related shifts in fish communities across an urban gradient to geomorphic change (Walters et al. 2003*a*) and to hydrologic alteration associated with imperviousness (Roy et al. 2005). It is possible that the mechanism by which urbanization causes extirpation varies from species to species. Indeed, considering the variability in natural-history characteristics among different fish species, we believe this is quite likely to be the case: some fishes will be sensitive to alteration of spawning habitat and others to toxins in their larval stage, while still others are feeding specialists that will respond to shifts in their food resources. If these responses were better understood, management strategies could be better targeted to the needs of individual species. However, there is consid-

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erable value in understanding the overall thresholds of responses of individual species, even without a mechanistic understanding, because these thresholds can be used to inform policies that broadly manage the impacts of urbanization. For example, most of the potential mechanisms are driven by stormwater runoff from impervious surfaces, which can be controlled by stormwater management programs designed to mimic natural hydrology (e.g., Ladson et al. 2006).

In previous studies in the Etowah basin, urban effects were found to be strongest on fish species classified as regional endemics or fluvial specialists (Walters et al. 2003*a*; Roy et al. 2005). Of the species examined here, *C. trichroistia*, *P. palmaris*, and *E. scotti* were included in both of these categories. Our results provide strong evidence that *C. trichroistia* and *P. palmaris* are indeed sensitive to urban stressors, but that occurrence of *E. scotti* is not strongly related to current land use. We also found that *N. leptacanthus* appears to be influenced by impervious cover, despite the fact that this species had previously been included among cosmopolitan species (i.e., species which as a group responded neutrally or positively to urban impacts; Walters et al. 2003*a*). These exceptions serve as a reminder that species groupings based on traits and classifications — such as endemics and cosmopolitans, or any of various index of biotic integrity metrics — may contain considerable noise in the form of species that respond in a manner opposite to what is expected. While such groupings may provide some utility in assessing the degree of impact experienced at the level of the fish assemblage, the response of a group as a whole cannot be used as a surrogate for the response of an individual species. However, it is realistic to use groups as a preliminary screen to identify species that are potentially sensitive to urbanization.

Similarly, general ecological correlates of imperilment such as benthic habit and small body size (Burkhead et al. 1997; Reynolds et al. 2005) may also be indicators of potential urban sensitivity. While such characteristics may provide an initial group of candidate species to test for sensitivity, they do not provide much insight into the degree of sensitivity. For example, the life-history traits of the highly sensitive *C. trichroistia* as a small fluvial specialist do not differentiate it from many other minnow species that are robust to urbanization effects.

Ultimately, effective management of the impacts of urbanization requires an understanding of the thresholds of response of individual species. For the two federally protected species we evaluated, we found good evidence for a strong response of *E. etowahae* to impervious cover but little evidence to indicate a relationship between presence of *E. scotti* and urbanization. These findings are consistent with field observations: *E. etowahae* is unknown from urban or suburban streams, while *E. scotti* has been collected in a number of suburban and moderately urban watersheds, albeit at lower abundances than in streams in forested or agricultural catchments. These results have important management implications, as they suggest that aggressive stormwater management policies are justified by the presence of *E. etowahae* but not by the presence of *E. scotti*.

We assert that in evaluating the sensitivity of individual species to urbanization or other hypothesized stressors, it is important to consider alternative hypotheses. Specifically, historic land use is likely to be of considerable importance in explaining present distribution patterns of many fish species. The extent to which this is true will depend on the magnitude of the historic effect and subsequent recolonization. Recolonization will depend on (1) persistence of effects or degree of habitat recovery, (2) movement potential and propensity of the species, and (3) presence of movement barriers. These factors may be difficult to assess. However, we suggest that in many cases it will be possible to use the type of approach presented here, or a simplified version, to compare the relative support for historic land use, current land use, hydrogeomorphic factors, or a combination of the three in explaining present fish distribution patterns.

This is the first study we know of to quantify the response of individual fish species to both historic and current land use. We found that both factors were important in explaining the distribution of fishes in an urbanizing watershed, and that some species exhibited a strong relationship to impervious cover, with occurrence probability approaching zero at levels above 2% EIA. Assessment of the response of individual species is a necessary step if we are to advance from merely cataloguing urban effects on fish assemblages to developing management policies that prevent extirpation of sensitive fish species.

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Quantifying invasion pathways: fish introductions from the aquarium trade

Erin Gertzen, Oriana Familiar, and Brian Leung

Abstract: Introduced species can cause economic and environmental harm. Researchers have developed risk assessment models for exotic species based on biological characteristics. However, few have quantified propagule pressure despite its relevance for establishment. Both are needed to identify invasion risk. We focused on fishes introduced via the aquarium trade, because this pathway transports thousands of species throughout the world. We developed an approach to estimate propagule pressure by (*i*) identifying and quantifying aquarium fishes sold, (*ii*) determining fish owner behavior and disposal practices, and (*iii*) quantifying uncertainty. We used the St. Lawrence Seaway as our model system. Only one non-established species (*Tanichthys albonubes*, 117 per year) had the propagule pressure and environmental tolerances to likely invade this region. However, overall, more than 10 000 fishes were released annually from Montréal (Quebec, Canada) alone. The implication of the observed propagule pressures is that the aquarium trade should be a very important pathway in other warmer habitats and should be explicitly assessed. Knowledge of the numbers introduced of each species will be useful for population models to estimate the probability of establishment.

Résumé : Les espèces introduites peuvent causer des torts économiques et environnementaux. Des chercheurs ont mis au point des modèles d'évaluation des risques associés aux espèces exotiques basés sur les caractéristiques biologiques. Cependant, peu ont quantifié la pression des propagules malgré l'importance du phénomène pour l'établissement de l'espèce introduite. Les deux types de données sont nécessaires pour évaluer le risque d'invasion. Nous nous sommes intéressés aux poissons introduits par le commerce de l'aquariophilie, car cette voie entraîne le transport de milliers d'espèces partout dans le monde. Nous avons mis au point une méthodologie pour évaluer la pression des propagules (*i*) en identifiant et quantifiant les poissons d'aquarium vendus, (*ii*) en déterminant le comportement et les pratiques de mise au rebut des propriétaires de poissons et (*iii*) en quantifiant l'incertitude. Nous utilisons la voie maritime du Saint-Laurent comme système modèle. Seule une espèce non établie (*Tanichthys albonubes*, 117 par année) possède la pression des propagules et les tolérances environnementales nécessaires pour vraisemblablement envahir la région. Cependant, en totalité, plus de 10 000 sont libérés chaque année seulement à Montréal (Québec, Canada). Les conséquences découlant des pressions des propagules observées sont que le commerce de l'aquariophilie devrait être une voie très importante dans d'autres habitats plus chauds et devrait être évalué de façon explicite. Une connaissance des nombres de poissons introduits de chaque espèce devrait être très utile pour construire des modèles démographiques pour estimer les probabilités d'établissement.

[Traduit par la Rédaction]

Introduction

In a world increasingly dominated by international trade, many biological organisms have either intentionally or accidentally been spread beyond their natural range. Human activity has facilitated the spread of species and accelerated the rate of introduction of nonindigenous species (NIS) into new environments (Mills et al. 1992; Hochberg and Gotelli 2005). The consequences are serious and include economic damage (Levine and D'Antonio 2003; Pimentel 2005) as well as decline in ecosystem function and decrease in global biodiversity (Miller 1989; Pimentel 2005). In the United States, costs associated with NIS are estimated at 137 billion dollars (US) annually (Pimentel et al. 2000). Now NIS are considered to be one of the most important factors in the loss of global biodiversity, second only to habitat loss (Wilcove et al. 1998; Levine and D'Antonio 2003).

Quantifying the risk posed by NIS is of utmost importance for their proper management (Ricciardi and Rasmussen 1998). Overall, rigorous risk assessment requires the integration of knowledge from different steps in the invasion process: transport, introduction, establishment, spread, and impact (Kolar and Lodge 2001). Introduction is a necessary first step in the invasion process, without which establishment, spread, and impact are impossible (Kolar 2004). Propagule pressure, or numbers introduced, is one of the best indicators of invasion success (Williamson 1996), with the likelihood of successful invasion being directly corre-

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lated to the number introduced (Kolar and Lodge 2001). To date, rates of introduction have often been neglected in risk assessment models — previous studies mainly focus on risk assessment based on biological characteristics of species (Mandrak 1989; Kolar 2004; Copp et al. 2005) and previous invasion history (Ricciardi and Rasmussen 1998). Very few studies have attempted to quantify the numbers of each species being introduced (but see Rixon et al. 2005). Ideally, we should quantify propagule pressures and integrate this with information on biological characteristics that will affect the ability of a species to establish (e.g., broad environmental tolerances) and spread (e.g., rapid growth rates) to cause harm.

Determining high risk species and pathways will allow us to focus limited resources where they are most needed and will be most effective. Prevention is considered to be one of the most effective ways of managing NIS (Lodge et al. 2006). For prevention to be successful, we need to know which species and how many individuals are being introduced by different pathways (Ricciardi and Rasmussen 1998; Kolar and Lodge 2002; Kolar 2004). Failure to identify important pathways may negate other investments in prevention efforts. For instance, vectors of introduction such as ballast water (Ricciardi and Rasmussen 1998; Rixon et al. 2005) have been the focus of a great deal of research. This research has lead to current regulation requiring mid-oceanic exchange of ballast water as a management tool to control the influx of ballast NIS (Ricciardi and MacIsaac 2000). However, other introductory pathways, including aquarium release, escape from aquaculture facilities, the live food market, intentional introduction, and live bait release (Mills et al. 1992), need to also be considered. Intentional release from the aquarium trade is an important pathway for the spread of NIS (McDowall 2004), with species from the aquarium and ornamental trade being responsible for one-third of the world's aquatic NIS (Padilla and Williams 2004). Moreover, there is little regulation of the aquarium trade in terms of restricting potentially nuisance NIS (Ricciardi and Rasmussen 1998; McDowall 2004; Rixon et al. 2005).

Despite its clear relevance, quantitative estimates of propagule pressures via different pathways are typically lacking. The major difficulty is that it is likely impossible to directly measure propagule pressure. However, it should be feasible to quantify the steps leading to an introduction. For instance, for the aquarium trade, the steps are as follows: species need to be for sale in stores (Rixon et al. 2005), customers need to purchase the fishes and dispose live fishes, and the disposal pathway needs to be in connected waterways to water bodies of interest (e.g., the Great Lakes). The number of fishes released may be non-negligible, as some individuals consider live release to be the most humane method of disposal (Courtenay 1999; Severinghaus and Chi 1999). Of course, the actual propagule pressure will depend upon how many of each fish species is bought and what proportion of those fishes are released. Some fishes are more likely to be disposed of than others. For instance, becoming bored with a fish, the ability of a fish to grow to a large size, and aggressive behavior have been identified as factors that increase the probability of disposal (Duggan et al. 2006).

In our study, we focused on four aspects related to the aquarium trade: (*i*) identifying and quantifying the fish species sold to customers in the aquarium trade, (*ii*) determining fish owner behavior, (*iii*) quantifying the uncertainty in our empirical estimates, and (*iv*) integrating the empirical data and uncertainty into a model to quantify propagules pressure. We used Montréal and the St. Lawrence Seaway as our model system.

Materials and methods

Study system

We focused on Montréal and the St. Lawrence Seaway, given its importance as a major entry point of NIS into the Great Lakes through a series of built shipping canals and locks (Environment Canada 1996) and potentially into other areas of North America (Northeast–Midwest Institute and National Oceanic and Atmospheric Administration 2001). The Great Lakes themselves experience damages estimated at 5.7 billion dollars (US) annually in control measures and losses associated with depletions of commercial and sport fish stocks owing to nuisance NIS (Pimentel 2005). Introductions via the aquarium pathway may be very important for this region, with over 5000 fish species, most of which are non-native to the Great Lakes basin, traded internationally in aquarium trade, and brought into the region (Welcomme 1984; Chapman et al. 1997; McDowall 2004). We focused on the island of Montréal, Quebec, Canada (45°28′N, 73°45′W) — the major metropolitan center adjacent to the St. Lawrence Seaway. Quantification of propagule pressure from Montréal will likely provide the most important source of propagules from the aquarium trade to the St. Lawrence Seaway.

Data collection

We used a combination of social surveys and aquarium fish surveys to estimate propagule pressure.

Store inventories

In this step of our study, we extended work by Rixon et al. (2005), who use number of stores carrying a given fish species as a surrogate for propagule pressure. Here, we quantified the number of individuals of each species sold. We visited >75% of aquarium and pet stores (18 stores) carrying fishes in Montréal from February to May 2006. The remaining stores were either unwilling to participate or unable to provide us with the required information. The fish species present in these remaining stores were verified to ensure that no species would be missing from our comprehensive list of species present on the island of Montréal. The information collected in this section was used to estimate relative propagule pressures of fish species rather than absolute numbers, which was estimated using customer surveys described below. We are aware that intra-annual variability in sales exists (Chapman et al. 1997); however, currently this is the best data we have available. The 4-month sample period of our surveys will take into account some of this variability, and it improves on previous work that looked at a 1-day occurrence of species sold in aquarium and pet stores (Rixon et al. 2005).

In each participating store, we determined the number of fishes sold over a month-long period, given by number sold

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in 30 days for species x = first count + sum of deliveries – final count. Because most stores simply record dollar value amounts and not species or quantities sold, we recorded standing stock populations in both the front and back rooms of the stores for every freshwater fish species at the beginning and end of the inventory period. To take into account new fishes that were delivered to the stores over the study period, we obtained copies of all relevant deliveries from store owners. Mortality was assumed to be of equal proportions between species and was not included in our analysis of relative species numbers. Several species were grouped together into genera (e.g., *Corydoras* spp.) or families (e.g., certain members of the Cichlidae family). This was necessary because we were limited owing to the fact that many of the deliveries simply stated "assorted corydoras," or "African cichlids."

Store owner surveys

We surveyed 20 aquarium and pet store owners and asked them to explain their policy on dealing with unsold fishes. The store owners surveyed included all stores where store inventories were conducted and two others. The aim of this component of the survey was to assess the likelihood of live release from aquarium and pet stores and to verify whether or not release directly from stores should be considered as a potential pathway contributing to the overall propagule pressure of aquarium fishes. We found that all fishes were either kept and put on sale until sold or returned to the distributor. Because none released fishes into the wild, store owners were not considered further.

Customer surveys

We conducted interviews with 86 customers outside 11 aquarium and pet stores in Montréal during October and November 2005. The purpose of the survey was to evaluate the history of behavior fish owners have had with their aquarium fishes. The survey instrument provided (*i*) the number of fishes owned in their lifetime and the time period over which they owned the fishes; (*ii*) the number of people who have released at least one fish in their lifetime; (*iii*) the proportion of fishes that had been released to the wild, returned to the store, given away, flushed down the toilet, or died in the aquarium; and (*iv*) the proportion of fishes released for a specific reason (aggressive behavior, large size, fish illness, rapid reproduction, becoming bored or annoyed with the fishes, not having time, or moving away). The survey was conducted in person and anonymously in efforts to ensure truthful and complete responses. From this data, we inferred the following: (*i*) the average number of fishes kept over a year (N), (*ii*) the probability that a person was a releaser ($P(I)$), (*iii*) the probability that a fish would be released given that the person owning it was a releaser ($P(R|I)$), and (*iv*) the relative probability that a fish was released, based on reasons for release (r_x). These variables were used in our model to generate propagule pressure.

Model for total propagule pressure

The total propagule pressure for all freshwater aquarium fishes from the aquarium trade is described by

$$\text{(1)} \qquad \text{overall propagule pressure} = M \cdot P(I) \cdot N \cdot P(R|I)$$

where M is the number of households that own fishes, $P(I)$ is the probability that a person is a releaser, N is the average number of fishes owned, and $P(R|I)$ is the probability that a fish is released given it is owned by a releaser.

The value for M was derived from existing literature (Chapman et al. 1997), whereas the values for $P(I)$, N, and $P(R|I)$ were calculated using data collected from customer surveys. We used a Bayesian statistical approach to assemble the components of the model for propagule pressure, taking into account uncertainty (i.e., extrapolating to the island of Montréal from our sample of 86 fish owners). A Bayesian approach was used to take into account the uncertainty of our data, given our sample size. Previous studies have not considered customer behavior as a factor affecting release, and we believe this is an important first attempt at doing so. Given we had no prior beliefs, we used an improper uniform prior such that the results obtained fully reflected our data. For example, if 7 out of 100 people sampled release fish, the most likely proportion of people releasing fishes in the population would be 0.07, given the data. However, there is a probability that the true population value was 0.06, and we observed 7 out of 100 people releasing fishes. We considered the probability of each true population value in our analysis, given the data observed. Probability distributions were generated for the components $P(I)$, N, and $P(R|I)$ (M was a constant). We multiplied the three distributions together — we considered all combinations of values for these three components and multiplied the probability that each value was correct, given the data, to create a joint probability distribution of our relative beliefs in numbers released. The joint probability distribution was our estimate, including uncertainty, of total propagule pressure of all fishes originating from aquarium and pet stores.

$P(I)$ is a binary variable; therefore, we used a binomial distribution. For the purpose of this study, a releaser is defined as a person who has released at least one fish into the wild over the time period in which he or she had kept aquarium fishes when interviewed. For the application of the model to the island of Montréal, we defined "wild" as the St. Lawrence Seaway. This assumption is justified because on the island of Montréal, the most likely water body in which a fish could be released in is the St. Lawrence Seaway. We recognize that it is possible that some fishes were released elsewhere on the island, such as in ponds; however, this is the best estimate we have.

$M \cdot P(I)$ gives the total number of releasers. To determine the number of fishes owned by releasers, we multiplied $M \cdot P(I)$ by N, which came from our survey data. We asked customers how many fishes they owned in their lifetime and the time period over which they owned these fishes. The nature of our surveys questioned people over their entire fish-owning history; thus, we took N as a yearly average of fish owned over the time people owned fish. We considered N as a yearly average over the fish-owning history of respondents and applied this average to Montréal's present population. Because of highly skewed data (most people owned few fishes and few people owned many fishes) ranging from 0 to 800, a lognormal distribution was used to describe the distribution of numbers of fishes owned.

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With the use of data for $P(R|I)$, the equation was further broken down by the proportion of fishes released out of total number of fishes owned by releasers. $P(R|I)$ represents the proportion of fishes released given they are owned by a releaser. Along with asking the method in which fishes were disposed of (released to wild, put in the garbage, given away, etc.), we asked fish owners the number of fishes that they disposed of over their fish-owning life and converted this into a yearly proportion. Possible values for $P(R|I)$ ranged from 0 to 1; therefore, we used the beta distribution to describe the distribution of $P(R|I)$ across releasers (i.e., we did not expect all individuals to release the same proportion of their fish). The multiplication of all these elements, $M \cdot P(I) \cdot N \cdot P(R|I)$, generated an estimate of total propagule pressure for all freshwater aquarium fishes from the island of Montréal.

Model for species-specific propagule pressure

The basic model for propagule pressure (eq. 1) was further broken down to estimate the propagule pressure of individual species of fishes by taking into account specific characteristics and population size:

(2) species-specific propagule pressure $= M \cdot P(I) \cdot N \cdot P(R|I) \cdot r_s \cdot c \cdot s_s$

where r_s is the relative rate of release, depending on specific characteristics, c is a correction factor, and s_s is the relative proportion of fishes belonging to a certain species. We estimated s_s using the total sold for a given species, summed across all stores during a standardized time period of 30 days.

Based on customer survey results, probability of release was adjusted for characteristics that affect this probability of release. Two characteristics, large size (defined as able to grow to 20 cm and over) and aggressive behavior, were common responses as reasons for fish release. Aggressiveness and maximum size of fishes were determined using www.fishbase.org/home.htm (Froese and Pauly 2005). All fish species were divided into four exhaustive and mutually exclusive categories, based on the two characteristics.

The variable r_s describes the relative increase in probability of release due to unwanted characteristics compared with reasons independent of fish characteristics (base rate = 1). We can calculate r, r_{as}, r_s, and r_a for different characteristics, based on T, T_{as}, T_s, and T_a, which are the proportion of fishes released for base reasons, aggressiveness or size or base reasons, size or base reasons, and aggressiveness or base reasons, respectively. The equations used to calculate the relative increase in rates of release due to undesired characteristics are as follows:

(3) base rate of release (nonaggressive and non-large species) : $r = T/T$

(4) relative increase in rate of release for species that can be both aggressive and large : $r_{as} = (T + T_a + T_s)/T$

(5) relative increase in rate of release for species than can be large but not aggressive : $r_s = (T + T_s)/T$

(6) relative increase in rate of release for species that can be aggressive but not large : $r_a = (T + T_a)/T$

These only provide relative rates of release. To determine the actual rates of release, a correction factor (c) was required such that summing species-specific propagule pressures (eq. 2) across all fish species would be equal to the total number of fishes released (eq. 1). To calculate c, we had to consider release rates based on species-specific characteristics (r_s) as well as total relative sales (F, F_{as}, F_s, F_a) of each category of fishes (none, both aggressive and large, large, and aggressive, respectively):

$$(7) \quad c = \frac{1}{(F \cdot r) + (F_{as} \cdot r_{as}) + (F_s \cdot r_s) + (F_a \cdot r_a)}$$

Once $r_s \cdot c$ for the four categories was determined, the adjusted relative rates of release were multiplied by each s_s. According to its characteristics and relative population size, the propagule pressure of each fish species was calculated using the model with the appropriate r_s and s_s values for that species (eq. 2).

Results

Store inventories

Store inventories revealed that 252 species and a total of 46 722 fishes were sold over a 30-day period in the 18 stores we sampled. The top five species sold (Table 1) were goldfish (*Carassius auratus*), guppies (*Poecilia reticulata*), assorted platyfishes (*Xiphophorus* spp.), neon tetras (*Paracheirodon innesi*), and mollies (*Poecilia sphenops*). Goldfish, neon tetras, and Siamese fighting fish (*Betta splendens*) were the only species present in 100% of stores (frequencies of occurrence); however, sales of goldfish (23.2% of total sales) were much higher than sales of neon tetras (8.5%) and Siamese fighting fish (2.4%).

Customer surveys

Customer surveys revealed that 6 out of 86 respondents (6.98%; $P(I)$) reported having released at least one fish (Table 2). The average number of fishes owned per year was five, based on the lognormal distribution. The average percentage of fishes released, derived from customer survey data on numbers released given numbers owned by a releaser, was 5.1% ($P(R|I)$).

The reasons for which fishes were unwanted were used to determine the differential rates of release for the four mutually exclusive categories of fish (large size, aggressive, both large size and aggressive, and none). Customer surveys

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Table 1. Relative numbers sold, propagule pressure (with and without characteristics of large size and aggressive behavior taken into account), temperature tolerances, and behaviors for the 30 most popular freshwater aquarium fish species.

		Population		Propagule pressure				
Common name	Scientific name	Relative size	With character adjustment	Based on relative population size	With character adjustment	Lower temperature tolerance (°C)*	Aggressive*	Large size*
Goldfish	*Carassius auratus*	0.2317	0.2537	2340.5	2562.8	0		X
Guppy	*Poecilia reticulata*	0.0849	0.0799	857.3	807.5	18		
Assorted platyfishes	*Xiphophorus* spp.†	0.0499	0.0511	519.7	489.5	18	X	
Neon tetra	*Paracheirodon innesi*	0.0514	0.0485	503.7	515.9	20	X	
Molly	*Poecilia sphenops*	0.0353	0.0332	356.2	335.5	18		X
Zebra danio	*Danio rerio*	0.0295	0.0278	298.3	281	18		X
Assorted swordtails	*Xiphophorus* spp.†	0.0285	0.0292	287.7	294.7	22		
Siamese fighting fish	*Betta splendens*	0.0239	0.0245	241.6	247.5	24	X	
Tiger barb	*Puntius tetrazona*	0.0227	0.0213	228.9	215.6	20	X	
Assorted plecostomus	*Pterygoplichthys* spp.	0.0127	0.0139	228.8	209.9	18	X	
Coolie loach	*Pangio kuhlii*	0.0184	0.0173	185.4	174.6	24		
Cardinal tetra	*Paracheirodon axelrodi*	0.018	0.017	181.9	171.3	23		X
Corydoras catfish	*Corydoras* spp.	0.0177	0.0167	179.3	168.8	18	X	
Angelfish	*Pterophyllum scalare*	0.0177	0.0167	178.9	168.5	15.5		
Black neon tetra	*Hyphessobrycon herbertaxelrodi*	0.0151	0.0142	152.3	143.5	23		
Harlequin rasbora	*Trigonostigma heteromorpha*	0.0138	0.013	128.1	140.2	22	X	X
White cloud mountain minnow	*Tanichthys albonubes*	0.0123	0.0115	123.8	116.7	5		
Dwarf gourami	*Colisa lalia*	0.0102	0.0096	103.1	97.1	22		
Chinese algae-eater	*Gyrinocheilus aymonieri*	0.0087	0.0095	100.2	94.4	22		
Danio burmese glowlight	*Danio choprai*	0.0099	0.0093	97.8	92.1	20		
Norman's lampeye	*Aplocheilichthys normani*	0.0097	0.0091	87.7	82.6	22		X
Clown loach	*Botia macracanthus*	0.0075	0.0089	87.5	95.8	24	X	
Lemon tetra	*Hyphessobrycon pulchripinnis*	0.0087	0.0082	80.4	75.7	22.5		
Black phantom tetra	*Megalamphodus megalopterus*	0.008	0.0075	77.8	73.3	22		X
Bloodfin tetra	*Aphyocharax anisitsi*	0.0077	0.0073	76.2	89.7	18		
Glowlight tetra	*Hemigrammus erythrozonus*	0.0072	0.0068	72.9	68.7	24		
Blackline penguinfish	*Thayeria boehlkei*	0.0067	0.0064	68.2	64.2	22		
Jewel tetra	*Hyphessobrycon eques*	0.0064	0.0066	66.3	64.7	22	X	X
Golden otocinclus	*Otocinclus affinis*	0.0064	0.006	64.7	61	20		
Firehead tetra	*Hemigrammus bleheri*	0.0063	0.0059	63.6	59.9	23		

*Based on Froese and Pauly (2005).

†*Xiphophorus* spp. is used for platyfishes and swordtails to describe two groups of visibly distinguishable assortments of species.

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Table 2. Responses to customer survey questions (n = 86).

Question	Percentage of respondents
Percentage releasing fish ($P(I)$)	6.98
Percentage released by releasers ($P(R\|I)$)	5.10
Reason for disposal	
Aggressive behavior	7.04
Large size	13.03
Frequent illness	15.14
Rapid reproduction	1.06
Other	63.73

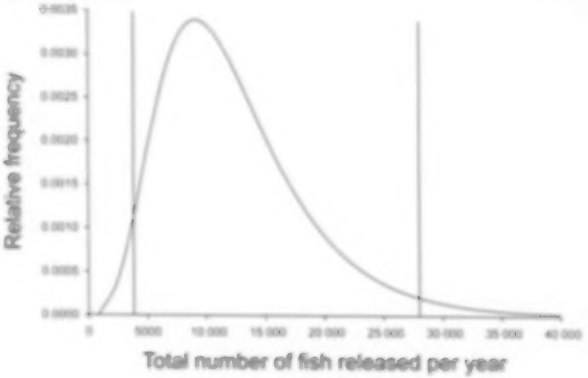
showed that 15% of unwanted fishes were unwanted because of fish illness, 13% because of large size, 7.0% because of aggressive behavior, and 1.0% because of rapid reproduction. The rest of the fishes were unwanted for reasons other than the characteristics of the fishes, including becoming bored with fishes or moving. Other reasons for release (e.g., fish illness and rapid reproduction) were considered as the base rate of release (r). Fish illness was incorporated into the base rate because illness is often dependent on the environment in which the fish lives (size of tank, cleanliness of water, density of fishes, etc.), more so than the characteristics of the fish species itself, and these fishes would likely die in the new environment if already unhealthy. Reproduction was likewise not included because they accounted for only 1% of release and because of the lack of data available on reproductive rates for the majority of aquarium fish species. The base rate accounted for 80% of total unwanted fishes. Fishes that have the ability to grow to a large size and aggressive behavior were assigned greater probabilities of release, based on their importance revealed in survey data. Using eqs. 3, 4, 5, and 6, r = 1, r_{ag} = 1.25, r_s = 1.1625, and r_a = 1.088.

Model for total propagule pressure

The framework developed here was used to find the propagule pressure of all fishes being released from the island of Montréal (eq. 1). The distributions were multiplied together, as well as by M applied to the present population of Montréal; the population of households on the island of Montréal (805 820; Statistics Canada 2001) was multiplied by 10.6% (percentage of North American households that own fishes; Chapman et al. 1997) and 96% (percentage of aquarium fishes of freshwater origin; Chapman et al. 1997) to give the total number of households that keep freshwater aquarium fishes — 82 002 households. These published figures were used to estimate M because we believe their universality provides better data of the number of households that own freshwater aquarium fishes than a simple snapshot of the population of freshwater aquarium fish owners in Montréal in one time period.

Our study was broken into two sections and data was obtained from each section: total number of fish owned (N) from customer surveys and proportion of fish species sold (s_i) from store inventories. We used customer survey data to estimate absolute numbers of fish owned, because we were more confident in these estimates than in estimates of total number of fishes sold in stores because of the seasonality issue.

Fig. 1. Relative probability distribution of propagule pressure of all fish from the island of Montréal. The mode of the distribution is 10 103.6, with a 95% certainty that more than 4600 fish are released per year.



We multiplied together the three posterior distributions, all from customer survey data (N, $P(R|I)$, $P(I)$), to give us total propagule pressure and the uncertainty around our data. The most likely propagule pressure was 10 103.6 fishes released per year. Based on the uncertainty associated with the data, there is a 95% chance that the true propagule pressure was at least as great as 3800 fish per year and lower than 27 900 fish per year (Fig. 1).

Propagule pressure by species

To generate propagule pressure by species, the outcome of the basic model was multiplied by $r_s \cdot c \cdot s_i$. Of all fishes sold, the proportion that were aggressive was 0.1267 (F_a), whereas 0.2970 could grow to be too large (F_s), 0.0092 were both aggressive and too large (F_{as}), and 0.5670 were neither aggressive nor grew to be too large (F). These values of F_a, F_s, F_{as}, and F were used in the equation to determine c, the scaling factor for relative rates of release (0.9419). The values for s_i, the relative population of all species, came directly from store inventory data. For example, $s_{goldfish}$ was 0.23 and s_{guppy} was 0.085 (Table 1). Using the model, we found that goldfish, a species that can grow to be large, has a propagule pressure of 2563 when we take into account characteristics and 2340 when we do not take into account characteristics. A histogram of release rates for all 252 aquarium fish species was created (Fig. 2). Rare and unpopular species have negligible propagule pressure (<1). This shows us that most fish species have low propagule pressure, whereas a few popular species have high propagule pressure.

Discussion

The knowledge acquired from risk assessment and propagule pressure models help us better understand and manage the introduction, establishment, and spread of potentially nuisance NIS. Understanding the importance of different pathways of introduction helps us to focus limited resources. Popular aquarium and ornamental trade species (e.g., goldfish and koi carp (i.e., domesticated varieties of the common carp, *Cyprinus carpio*)) have already established populations in the Great Lakes – St. Lawrence Seaway region (Mills et

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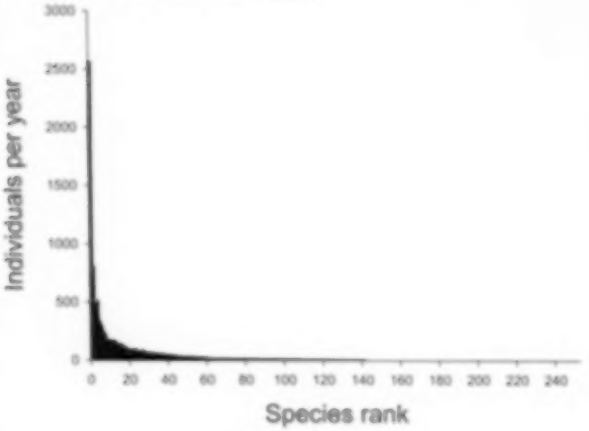
Fig. 2. Rank – propagule pressure curve for all fish species sold in Montréal aquarium and pet stores over the study period. The histogram shows that most species have low propagule pressure and few species have large propagule pressure.



al. 1992), suggesting that further investigation into the importance of the aquarium trade in importing potentially nuisance NIS may be merited.

The implications of propagule pressure are great. Propagule pressure is highly related to establishment success (Kolar and Lodge 2001). Organisms must enter a new environment in numbers great enough for reproduction and spread to occur. Moyle and Marchetti (2006) suggest that a propagule pressure of greater than 100 individuals for Californian lakes and streams is adequate for establishment. In our study, we observed that 18 species had propagule pressures exceeding 100, and 42 species had propagule pressures exceeding 40, but only two of these species (goldfish and koi carp) are known to have established populations in the St. Lawrence Seaway. Nevertheless, the number of colonizers required will depend on other factors affecting establishment. Propagule pressure required for establishment will vary depending on the size of the habitat area, frequency versus dose of introductions, and Allee effects (Leung et al. 2004). For example, for a population to establish, individuals must be able to find mates and reproduce. In a large system such as the St. Lawrence Seaway, it is likely that this would occur less than in smaller systems. Determining the required propagule pressures for individual species would require population dynamics models, which in turn would relate to species characteristics such as reproductive rate and mortality given physical and chemical conditions in the new habitat.

The establishment of most aquarium species in the St. Lawrence Seaway is likely mainly limited by extreme physical conditions, namely cold temperate waters. Most aquarium species are tropical in origin and have poor cold tolerance (see Table 1 for cold tolerance of top 30 species; Mandrak 1989; McDowall 2004; Duggan et al. 2006). Of all species sold, five non-native species (one native species was excluded) can survive at low temperatures (<5.5 °C; temperature requirement for the Great Lakes – St. Lawrence Seaway region, as defined by Rixon et al. 2005), two of which are already established. The other nonestablished, cold-tolerant NIS are the white cloud mountain minnow (*Tanichthys albonubes*), weatherfish (*Misgurnus fossilis*), and hogchoker (*Trinectes maculatus*); however, the weatherfish and hogchoker have very low propagule pressures (6 and 1 individuals per year, respectively). In our data, the white cloud mountain minnow was reported with a propagule pressure in the top 20, at 117 per year, highlighting the potential high risk associated with this species. Thus, based on our findings, we can greatly focus our research and management efforts in the St. Lawrence Seaway. The quantitative estimates of propagule pressure that we provide in this study will be useful for population dynamics models should researchers decide that further research is important. Specifically, while preliminary evidence from other systems suggests that 100 individuals per year should be regarded as high risk, we recommend that for white cloud mountain minnow, a population model explicitly including demographic and environmental stochasticity, Allee effects, mortality rates, and reproductive rates should be built to determine whether 117 fish introductions per year is sufficient for establishment in this system.

More generally, for the St. Lawrence Seaway, researchers and managers can focus their efforts on other pathways. However, the implications of the magnitude of overall propagule pressure observed in this study (over 10 000 individual fish), with 42 species having propagule pressures greater than 40, is that the aquarium trade should be important for other regions with different environmental conditions (i.e., the moderate waters of western Canada or the warm waters of Florida; McDowall 2004; Padilla and Williams 2004) and that propagule pressures should be quantified in other areas. For example, assuming similar numbers and species sold, in a warmer west coast city such as Vancouver (winter water temperature of 8 °C; Fisheries and Oceans Canada 2006), nine species could potentially establish. In a warmer southerly city such as Miami (winter water temperature of Lake Okeechobee = 19.4 °C; International Lake Environment Committee 2001), at least 55 species could potentially establish.

Although we believe that we provide the best current estimates of propagule pressure for aquarium species, the full story remains incomplete. This study focuses on one pathway of introduction into a major system (St. Lawrence – Great Lakes basin). A full analysis of invasion risk for this region would expand to look at all species and all pathways of introduction. Our study identifies a simple method to quantify propagule pressure, which can be applied both to other pathways (i.e., live bait or live food market) in the St. Lawrence Seaway and to the aquarium trade in other regions of the world that may be more susceptible to potentially nuisance NIS from the aquarium trade.

Our work extends previous work by providing quantitative estimates of numbers introduced. Previous studies suggest the importance of popularity, as estimated by frequency of stores selling a species, in determining propagule pressure (Rixon et al. 2005; Duggan et al. 2006). We extended these studies by determining the actual numbers sold and integrating that with other information available to quantify propagule pressure. Previous studies also suggest that large size and aggressive behavior are important factors determining the likelihood of aquarium release (Courtenay

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1999; Duggan et al. 2006). We investigated this further and were able to quantify their importance. Although 7% of releases were due to large size and 13% were due to aggressive behavior, these characteristics did not greatly alter species-specific propagule pressure (see Table 1 for propagule pressure before and after release rates were adjusted for these characteristics).

Further, our study focused on the fate of aquarium fishes that are sold in aquariums and pet stores. Although the overall number of fish introductions was based on the customer surveys and would remain unaffected, other factors could result in a higher propagule pressure for specific species than estimated in this study. One such area is reproductive rate. This focus excluded fishes originating from other means such as the Internet or breeding by hobbyists (Padilla and Williams 2004). For example, water hyacinth (*Eichhornia crassipes*), a nuisance aquatic plant that is banned in aquarium and pet stores in many regions, is still available on the Internet for only $4 (Padilla and Williams 2004). The potential impact of the Internet trade on imported species may be substantial and should be investigated along with more traditional avenues of trade in aquarium fishes. Similarly, our study did not cover the extent to which fishes are bred and distributed by hobbyists. Aquarium societies, growing in popularity, have fish auctions at which they sell fishes they have bred at home or that they can no longer take care of. Finally, we considered a 4-month period to represent an average sales amount for the year overall. Generally, this should be a good estimate of the relative numbers of fish sold. However, there may be intra-annual variation due to factors such as population dynamics of individual species, seasons, and religious holidays (Chapman et al. 1997; Severinghaus and Chi 1999) that were not considered. Further, differential mortality in stores may also cause errors in the estimates of fish sold.

Nevertheless, we advance the field by focusing on the behavior of fish owners as a major determinant of propagule pressure because it is ultimately humans who determine the rate at which aquarium fishes are entering our waterways. Fisheries and Oceans Canada states that the most effective method of controlling NIS is to regulate the pathway of introduction (Fisheries and Oceans Canada 2004). Therefore, modifying human behavior is the key to reducing the potential ecological and economic impacts of live aquarium release. Indeed, more educated people are less likely to participate in religious releases of fishes (Severinghaus and Chi 1999). Educational tools such as the Habitattitude campaign (www.habitattitude.net) already exist and should be implemented on a wider scale. Education of the general public could reduce overall rates of introduction and possibly lead to the mitigation of the damaging consequences of NIS.

In conclusion, our study provides an important first step into looking at how human behavior and fish popularity affect rates of introduction. We found that over 10 000 fishes are released annually from the Montréal aquarium trade to the St. Lawrence Seaway, with fish characteristics of aggressiveness and large size minimally affecting release rates. Although the establishment of most aquarium species is limited in this region owing to environmental tolerances, the framework and empirical data provided here can be used to estimate propagule pressure and invasion risk in other regions that may be more susceptible to invasions from the aquarium trade. Further research should attempt to combine studies on propagule pressure of aquarium fishes with risk assessment models based on biological characteristics and population dynamics to obtain a more rigorous estimate of invasion risk as a means to better focus management efforts.

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Genetic structure, migration, and patterns of allelic richness among coho salmon (*Oncorhynchus kisutch*) populations of the Oregon coast

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Abstract: Genotypic data from eight microsatellite loci are used to infer population structure, effective population size, migration rates, and patterns of allelic richness among wild and hatchery populations of Oregon coastal coho salmon (*Oncorhynchus kisutch*). Corroborating the results of a previous study, we found relatively weak genetic structure among coho from different river basins, although some geographically and ecologically defined clades are supported. Contemporary migration rates among basins appear to be high and asymmetrical. Hatchery populations tended to resemble the wild populations from which they were founded, but presented significantly lower levels of allelic richness. Allelic richness was also low in Oregon coastal lake populations and peaked in the central region of the evolutionarily significant unit among wild river populations. We suggest that the observed patterns may reflect both current source–sink dynamics and post-Pleistocene colonization events.

Résumé : Les données génotypiques provenant de huit locus microsatellites nous ont servi à déduire la structure de la population, la taille effective de la population, les taux de migration et les patrons de richesse allélique chez des populations sauvages et de pisciculture de saumons coho (*Oncorhynchus kisutch*) de la région côtière de l'Oregon. Nous trouvons une structure génétique relativement faible parmi les saumons coho des divers bassins versants, ce qui corrobore les résultats d'une étude antérieure; néanmoins, il y a évidence de quelques clades définis géographiquement ou écologiquement. Les taux de migration actuels d'un bassin à l'autre semblent être élevés et asymétriques. Les populations de pisciculture tendent à ressembler aux populations sauvages dont elles sont issues, mais elles affichent des taux significativement plus bas de richesse allélique. La richesse allélique est également basse dans les populations lacustres de la côte de l'Oregon et elle atteint son maximum dans la région centrale de l'unité évolutive significative parmi les populations sauvages d'eau courante. Nous pensons que les patrons observés sont le reflet à la fois de la dynamique actuelle de type source–drain et des événements de la colonisation après le pléistocène.

[Traduit par la Rédaction]

Introduction

The life histories of Pacific salmon (*Oncorhynchus* spp.) present unique challenges for management and conservation. For example, the tendency for adult salmon to return to their natal streams as they prepare to spawn is believed to isolate populations over a spatial scale and mediate genetic divergence of locally adapted stocks (Taylor 1991). Moreover, in some species, fixed maturation ages, combined with semelparity, limits matings among individuals from different brood years to events involving less abundant precocial individuals (jacks). For example, most coho salmon (*Oncorhynchus kisutch*) mature and spawn at age 3+ years, although a fraction return to spawn at age 2+ years. Consequently, temporally isolated subpopulations can occur within a single watershed.

Given the potential for population structure at multiple scales, the rapid decline of many Pacific salmon stocks in the late 1980s prompted managers and conservation biologists to consider demographic independence and genetic distinctiveness as key criteria for the establishment of management units (Allendorf and Phelps 1981; Waples 1991; Utter et al. 1993). Allozyme studies (e.g., Utter et al. 1973; Beacham et al. 1985; Weitkamp et al. 1995) provided the first source of genetic data for the delineation of the 52 Pacific salmon evolutionarily significant units (ESUs; Waples 1991) now recognized by the US Endangered Species Act. However, the statistical power provided by allozyme data to discriminate among populations varies greatly for Pacific salmon species (Utter 1991), limiting the general applicability of these markers.

Advances in molecular genetic technology have since allowed researchers to uncover previously undetected levels of genetic diversity within and among populations of Pacific

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salmon. For example, highly polymorphic microsatellite markers have elucidated significant genetic structure among coho salmon within and among river basins of California (Bucklin et al. 2007), Oregon (Ford et al. 2004), British Columbia (Small et al. 1998; Beacham et al. 2001), and Alaska (Olsen et al. 2003). Over a larger scale, Smith et al. (2001) used mtDNA sequence data and microsatellite markers to describe patterns of coho genetic diversity throughout the species' North American range. A latitudinal cline of mtDNA haplotype diversity led Smith et al. (2001) to hypothesize that early Pleistocene glaciations had reduced the North American distribution of coho salmon to southern refugia in California and (or) Oregon. Latitudinal clines of genetic diversity in other nearshore fishes have similarly been described (e.g., Adams et al. 2006; Gysels et al. 2004). Only three coho populations from California and Oregon were considered by Smith et al. (2001), thus limiting the resolution of analyses in this region.

Recently, Ford et al. (2004) used seven microsatellite loci to more thoroughly characterize the genetic structure of coho salmon populations from the Oregon coast. In addition to structure analyses, the authors tested for a signal of genetic introgression from an aquacultural operation, which utilized non-native broodstock on the central Oregon coast. Their findings generally supported previously hypothesized population complexes and clearly acknowledged the potentially confounding effects of anthropogenic activities over coho salmon genetic diversity.

In this study, we use data from eight microsatellite loci to characterize genetic structure within and among putative coho salmon populations from 23 river basins of the Oregon coastal coho (OCC) ESU, as well as a single basin (Rogue River) from the Northern California – Southern Oregon ESU (Fig. 1). We utilize temporally replicated samples of wild populations from five river basins to estimate the effective number of breeders, immigrant fractions, and the percentage of genetic variation apportioned among river basins, brood-years, and within basin sampling sites. Directional migration rates among wild coho populations are estimated through a Bayesian assignment method (Wilson and Rannala 2003), and in a final analysis, we examine latitudinal patterns of allelic richness among wild and hatchery coho populations of the Oregon coast.

Materials and methods

Sampling and genotyping

During the spawning seasons of 2002, 2003, and 2004, tissue samples were collected from expired, presumably wild origin coho salmon spawners from 24 coastal Oregon river basins. Wild origin fish were distinguished from hatchery coho through the presence of an intact adipose fin, which is removed from nearly all coastal Oregon hatchery coho prior to release (84%, 96%, and 97% marked for brood years 2002, 2003, and 2004, respectively). In 2004, samples were also collected from adipose fin-clipped coho returning to five Oregon hatcheries. Tissue samples were preserved in 95% ethanol, and the collection location and date were recorded together with the length and sex of each fish sampled. From the 2002 collection, samples from all rivers were included in our analyses. Samples from four basins in the 2003 collection (Nehalem, Yachats, Smith, and Coos) and five basins in the 2004 collection (Nehalem, Yachats, Smith, Coos, and Coquille) were also included in our analyses. Genomic DNA was extracted through DNEasy (Qiagen Inc., Valencia, California) or Chelex – proteinase K (Estoup et al. 1993) protocols, and separate polymerase chain reactions were carried out in 5 μL volumes on a MJ Research thermocycler to amplify eight microsatellite loci, utilizing fluorescently labeled primers (*One13*, Scribner et al. 1996; *Ots2*, Banks et al. 1999; *p53*, de Fromentel et al. 1992; *Oki16*, Smith et al. 1998; *Ots215*, Greig et al. 2003; *Ots520*, Naish and Park 2002; *Ots3*, Banks et al. 1999; *Oc18*, Condrey and Bentzen 1998). PCR products were separated via polyacrylamide gel electrophoresis on an ABI 3730XL genotyper and (or) MJ Research Basestation and binned according to size with either GeneMapper or Cartographer software. A minimum of 96 samples were analyzed at all loci on both genotyping platforms to allow for bin adjustments and consistent size scoring.

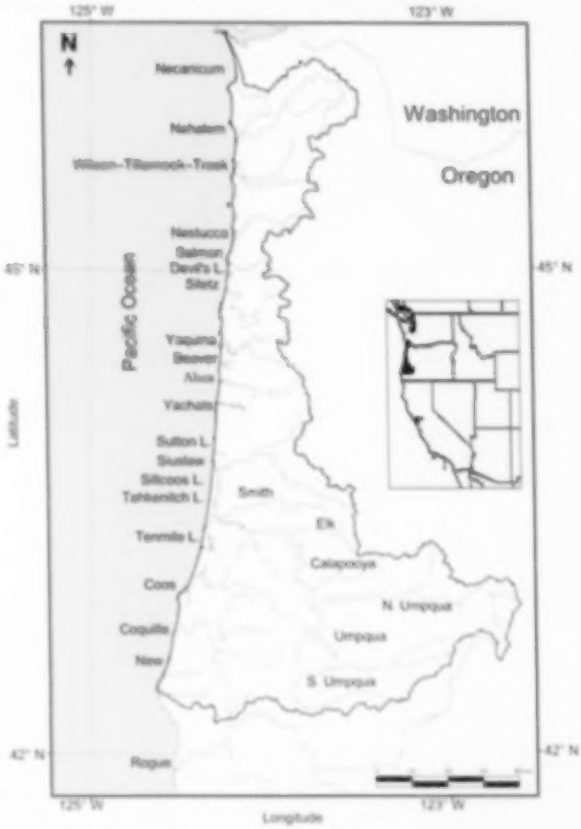

Fig. 1. Map of rivers from which tissue samples were collected. The Oregon coastal coho evolutionarily significant unit is outlined in bold. Inset shows location on the US west coast.

Statistical analyses

We used the program FSTAT (Goudet 1995) to calculate the per-locus and overall heterozygosity for each population, as well as performed permutation tests (1000 iterations) to

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detect departures from Hardy–Weinberg equilibrium (HWE). The program GENETIX (Belkhir et al. 2004) was used to carry out permutation tests (1000 iterations) to detect linkage disequilibrium among loci. Sequential Bonferroni corrections (Rice 1989) were made to adjust the initial critical value of 0.05 to account for multiple comparisons made during these tests.

Pairwise values for Weir and Cockerham's inbreeding coefficient, θ, were calculated for all wild 2002 samples with the program GENETIX (Belkhir et al. 2004), and we used a permutation test with 1000 iterations to assess the statistical significance of these estimates. Similarly, we calculated all pairwise θ values for all major tributaries of the Umpqua River to assess structure within this basin. We used the program GDA (Lewis and Zaykin 2001) to perform an analysis of molecular variance (AMOVA) on data from four temporally replicated populations (Nehalem, Yachats, Smith, and Coos), simultaneously estimating the percentage of total genetic covariance explained by allele frequency differences among river basins, years, and within-basin sampling sites for each year.

We used the maximum likelihood phylogeny inference program, CONTML, in the PHYLIP analysis package (Felsenstein 2005) to construct trees depicting the structure of coho salmon genetic diversity among all 24 basins sampled in 2002. Five hatchery populations and temporal replicates (2003, 2004) of wild populations from five river basins were also included in this analysis. We used the program SEQBOOT, also in the PHYLIP package, to bootstrap the data and estimate statistical support for the topology of the best maximum likelihood tree. We displayed trees with the program TREEVIEW (Page 1996).

For the five populations that were sampled in multiple years, the number of breeders, N_B, was estimated through the methods of Waples (1990):

$$\hat{N}_B = \frac{b}{2(\hat{F} - 1/S)}$$

where $\hat{F}$ is an estimator of the standardized temporal variance in allele frequencies, $1/S$ is a sampling error correction factor, and b is the number of generations between samples, adjusted to account for the relative contribution of jacks to the population. Values of b were calculated according to the methods of Tajima (1992). N_B was then used to estimate m, the per-population immigrant fraction, according to the temporal F_{ST} method (Ford et al. 2004).

Using only data from 2002, we applied a Bayesian method to estimate directional migration rates among wild coho salmon populations. This method, described by Wilson and Rannala (2003) and implemented in the program BAYESASS, relaxes several assumptions carried in the temporal F_{ST} method (e.g., constant and symmetrical geneflow between population pairs, HWE) and is designed to provide an estimate of recent migration rates. By inferring the ancestry of each sample through Markov chain Monte Carlo methods, BAYESASS provides directional estimates of migration, which may be used to infer source–sink dynamics. However, computational limits of the program necessitated some data pooling. A minimum number of pooling steps were made, utilizing evidence for natural clades identified through the maximum likelihood inference of coho population structure. Briefly, (*i*) the lower Umpqua and neighboring coastal lakes were pooled into a single clade; (*ii*) all other Umpqua tributaries were pooled into a single clade; and (*iii*) the Wilson and Tillamook rivers were pooled together as a single clade. Also, the number of alleles present at the *Oki16* locus exceeded the computational limits of the program. Thus, data from this marker were not included in the analysis. Lastly, the current version of BAYESASS limits the maximum number of populations considered to 19. We therefore performed the analysis twice, first excluding the northernmost population (Necanicum) and then excluding the southernmost population (Rogue). We used the default prior values (allele frequency, migration rate, and inbreeding coefficient all set at 0.15) and performed three million steps (999 999 burn-in) with a sampling frequency of 2000.

Allelic richness, a measure of genetic diversity that accounts for variable sample sizes through rarefaction, was calculated for all loci in all populations with the program FSTAT (Goudet 1995). An analysis of variance, carried out with the program S-PLUS 7.0 (Insightful Corp., Seattle, Washington), was used to test for associations between each population's mean allelic richness (across loci) and the following explanatory variables: "latitude" of entry from the ocean into the spawning river (river mouth), sampling "year", "ecotype" (river or lake), and "origin" (hatchery or wild). All variables were treated as fixed effects, and all variables were categorical, except for latitude, which was continuous. Owing to the unbalanced design of our study (e.g., absence of lake hatcheries and temporal replication for only wild fish from rivers), we used type III sums of squares to assess the significance of variables included in our models, but could not estimate the significance of third- and fourth-order interaction terms. We included all first-order interactions, with the exception of ecotype × year (not estimable), in an initial full model, then manually removed insignificant terms to obtain our final reduced model. We then used linear regression analysis to test for a cline in allelic richness among wild coho populations, using the continuous explanatory variable latitude.

Results

The eight microsatellites we used to characterize Oregon coastal coho salmon populations presented high yet variable levels of polymorphism, with between 10 and 71 alleles (mean = 30.12, standard deviation (SD) = 18.52). Per-locus heterozygosities ranged from 85.2% to 60.2%, with a mean of 72.8% overall (Table 1). Even after sequential Bonferroni corrections, approximately 64% of the wild populations did not conform to HWE expectations across all loci, whereas only one of five hatchery populations differed significantly from expected HWE genotypic frequencies. After making sequential Bonferroni adjustments to an initial critical value of 0.05, we detected significant evidence for linkage disequilibrium for only 0.7% of locus pairs, considering all possible locus pair combinations across all populations.

Population structure

Pairwise θ values at the basin level ranged from 0.002 to

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Table 1. Data collected for all populations: mean sample sizes across loci, deviation from Hardy–Weinberg proportions (F_{IS}), observed and expected heterozygosities (H_O and H_E, respectively), latitude of river (at mouth), origin, and ecotype.

Basin	Mean *n*	F_{IS}	H_O	H_E	Latitude (°N)	Origin	Ecotype
Alsea	83.5	0.094	0.731	0.801	44.25	Wild	River
Beaver Creek	31.6	0.069*	0.733	0.774	44.31	Wild	River
Coos	178.5	0.099	0.729	0.807	43.21	Wild	River
Coquille	41.8	0.197	0.648	0.794	43.07	Wild	River
Devil's	37.3	0.110	0.669	0.741	44.57	Wild	Lake
Necanicum	30.0	0.115	0.696	0.771	46.01	Wild	River
Nehalem	162.1	0.089	0.708	0.775	45.40	Wild	River
Nestucca	55.4	0.089	0.714	0.776	45.09	Wild	River
New	42.8	0.055*	0.728	0.761	42.56	Wild	River
Rogue	44.4	0.092	0.672	0.730	42.25	Wild	River
Salmon	42.8	0.072	0.730	0.777	45.02	Wild	River
Siletz	46.4	0.120	0.685	0.769	44.54	Wild	River
Siltcoos	26.9	0.103	0.692	0.754	43.52	Wild	Lake
Siuslaw	130.6	0.161	0.683	0.811	44.00	Wild	River
Smith	90.5	0.109	0.722	0.805	43.40	Wild	River
Sutton	34.0	0.097	0.688	0.749	44.04	Wild	Lake
Tahkenitch	31.1	0.069*	0.725	0.765	43.48	Wild	Lake
Tenmile	57.6	0.073	0.739	0.789	43.33	Wild	Lake
Tillamook	13.3	0.082*	0.698	0.728	45.33	Wild	River
Trask	18.9	0.021*	0.750	0.744	45.33	Wild	River
Umpqua	275.25	0.074	0.757	0.816	43.40	Wild	River
Wilson	31.1	0.072*	0.739	0.782	45.33	Wild	River
Yachats	20.3	0.050*	0.770	0.789	44.18	Wild	River
Yaquina	111.6	0.166	0.668	0.796	44.37	Wild	River
Temporal replicates							
Coos 2003	54.6	0.093	0.731	0.798	43.21	Wild	River
Coos 2004	80.3	0.047*	0.775	0.808	43.21	Wild	River
Coquille 2004	44.9	0.055	0.723	0.757	43.07	Wild	River
Nehalem 2003	121.9	0.021*	0.790	0.803	45.40	Wild	River
Nehalem 2004	41.4	0.184	0.648	0.780	45.40	Wild	River
Smith 2003	58.0	0.079	0.734	0.789	43.40	Wild	River
Smith 2004	67.5	0.077	0.740	0.794	43.40	Wild	River
Yachats 2003	35.0	0.060*	0.768	0.803	44.18	Wild	River
Yachats 2004	33.9	0.073*	0.732	0.777	44.18	Wild	River
Hatcheries							
Coos 2004	91.0	0.005*	0.797	0.789	43.21	Hatchery	River
Coquille 2004	47.1	0.007*	0.794	0.780	43.07	Hatchery	River
Cow Creek (South Umpqua) 2004	42.8	0.027*	0.806	0.818	43.40	Hatchery	River
Nehalem 2004	28.3	0.100	0.703	0.764	45.40	Hatchery	River
Salmon 2004	50.0	0.002*	0.750	0.741	45.02	Hatchery	River
Total	2434.0						

Note: Samples were collected in 2002, except where indicated.

*No significant difference from Hardy–Weinberg equilibrium (HWE) after sequential Bonferroni correction.

0.068, with an overall θ value of 0.021 (95% confidence interval (CI) = 0.016, 0.027; $p < 0.0001$). Highest values tended to occur between the northerly Trask population and those of rivers and coastal lakes from the southern extreme of the ESU (see Appendix A, Table A1). Within the Umpqua River, pairwise θ values ranged between 0.004 to 0.030, with an overall value of 0.015 (95% CI = 0.010, 0.022; $p < 0.0001$). Highest pairwise θ values within this basin occurred between the Smith River and south fork Umpqua populations, followed by values associated with the lower Umpqua and all other Umpqua tributaries. For the Nehalem, Yachats, Smith, and Coos rivers, simultaneous hierarchal analyses indicated that 97.33% of the observed genetic variance could be attributed to differences among individuals. Most of the remaining 2.67% of genetic variance could be explained by allele frequency differences among basins (52.8%), followed by differences among sites within basins (29.5%), and lastly, differences observed among temporal replicates (17.7%).

In agreement with our analyses of genetic variance, the

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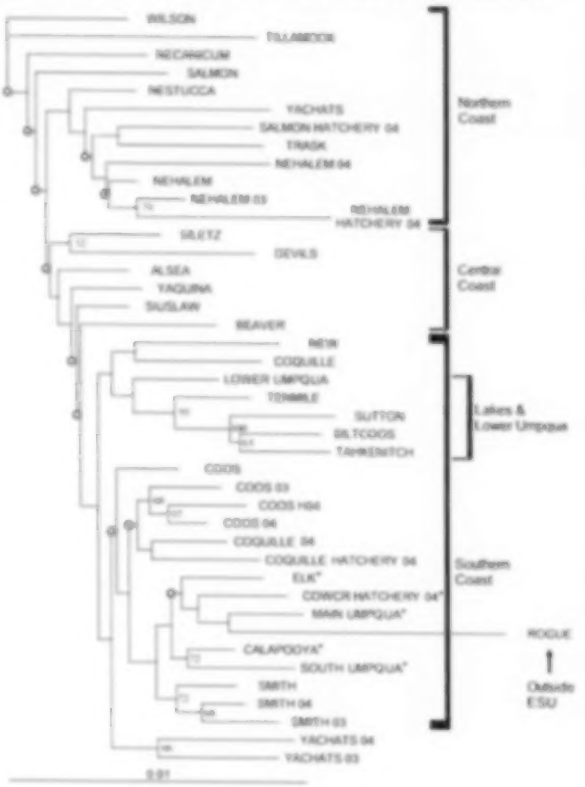
maximum likelihood tree inferred from our data suggests that only weak structure exists among coho salmon samples from different river basins of the OCC ESU, as many internal branch lengths are short or do not significantly differ from zero (Fig. 2). However, statistically supported clades generally reflect geographic relationships among basins. For example, the coastal lakes flanking the mouth of the Umpqua River form a clade together with the lower reaches of this river. Nearby Sutton Lake also falls into this group, whereas the more distant Devil's Lake does not, but instead appears to be most similar to its proximal Siletz River. The Coos, Umpqua, Smith, and Coquille rivers appear to form a southern group, whereas the Nehalem, Tillamook, Wilson, Necanicum, Nestucca, Trask, and Salmon rivers form a loosely defined northern group.

Samples collected from the same river in different years tended to form clades, although interannual Coquille samples alternately grouped with neighboring rivers to the north and south (Fig. 2). The Yachats River population presents another exception, as it does not fit neatly on the tree in a geographic context, but instead aligns with distant southern and northern populations between years.

In all but one case, hatchery coho salmon populations appear most similar to wild fish returning to the river where they are located (Fig. 2). The exception to this pattern is the Salmon River hatchery stock, which appears to be most similar to the Trask River wild population. Unlike other hatchery stocks considered here, the Salmon River hatchery population was not established from native fish from the river on which it operates. Instead it was founded by Siletz River hatchery stock (Salmon River Hatchery operations plan, Oregon Department of Fish and Wildlife, ODFW), which was heavily supplemented with Trask River coho during the years 1968–1977 (Beidler 1987). In concert, the pairwise θ value between the Salmon and Trask wild populations is the lowest observed between any two populations (Appendix A, Table A1), evidence that suggests that hatchery-mediated genetic introgression of Trask River stock may have occurred into the wild Salmon River coho population.

Fig. 2. Maximum likelihood tree depicting structure among Oregon coastal coho salmon (*Oncorhynchus kisutch*) populations. Open circles indicate branches of length not significantly different from zero ($\alpha = 0.05$). Values for nodes that received bootstrap support greater than 50% are presented. All samples were collected in 2002, unless otherwise indicated (03, from 2003; 04, from 2004). An asterisk (*) indicates a tributary or hatchery of the Umpqua River drainage.



Migration

Estimates of the effective number of breeders, based on the methods of Waples (1990), ranged from 66 to 384 individuals for the five populations examined (Table 2). Immigrant fraction estimates for these populations ranged from 0.02 to 0.14. The immigrant fraction point estimate in the Smith River, as calculated through the Bayesian assignment procedure implemented in BAYESASS (Wilson and Rannala 2003), exceeded those calculated through the temporal F_{ST} method of Ford et al. (2004) (Table 2), largely as a result of migration from the Umpqua and Lakes – Lower Umpqua migrant sources. In general, the Bayesian migration estimates tended to be higher, although estimates for the Nehalem River did not follow this pattern (Table 2). The Nehalem and Coos populations appeared to be the primary migrant sources for most basins, followed by the Umpqua and Lakes – Lower Umpqua (Appendix A, Table A2). Notably, our estimates for the Rogue River population indicate that strays from this basin account for less than 0.01% of the individuals present in any basin of the OCC ESU and that less than 0.01% of the Rogue population originated from any basin of the OCC ESU. This degree of isolation is not observed for any population within the OCC ESU. The BAYESASS program provided very consistent results between runs, with overlapping 95% CIs for all repeated estimates. Directional migration rate estimates among 20 population groups are presented in Appendix A (Table A2).

Allelic richness

Considerable variation in mean allelic richness was observed among Oregon coastal coho salmon populations. An analysis of variance indicated that first-order interaction terms for the explanatory variables year, origin, latitude, and ecotype were not significantly associated with variability in allelic richness ($p > 0.05$). Accordingly, the interaction terms were removed from the model, and a subsequent analysis of variance (ANOVA) identified both origin and ecotype to be significantly associated with variation in mean population allelic richness ($F_{[1,32]} = 4.430$, $p = 0.043$ and $F_{[1,32]} = 8.438$, $p = 0.007$, respectively; Table 3). Neither

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Table 2. Effective number of breeders (N_B) and corresponding immigrant fractions (m) for five Oregon coastal coho salmon (*Oncorhynchus kisutch*) populations as calculated by the temporal F_{ST} method (Ford et al. 2004), under 10% and 25% jack contributions.

	10% jacks		25% jacks		
Basin	N_B	m	N_B	m	Bayesian m*
Nehalem	170 (117, 245)	0.05 (0.02, 0.10)	76 (53, 110)	0.12 (0.06, 0.22)	0.06 (0.02, 0.10)
Yachats	150 (81, 348)	0.06 (0.02, 0.14)	66 (35, 153)	0.14 (0.05, 0.32)	0.32 (0.29, 0.33)
Smith	378 (210, 824)	0.02 (0.01, 0.06)	171 (95, 373)	0.05 (0.02, 0.12)	0.15 (0.06, 0.28)
Coos	384 (238, 674)	0.02 (0.01, 0.05)	172 (107, 301)	0.05 (0.02, 0.11)	0.19 (0.15, 0.24)
Coquille	168 (98, 324)	0.06 (0.02, 0.12)	71 (41, 137)	0.13 (0.05, 0.28)	0.31 (0.28, 0.33)

Note: For all estimates, 95% confidence intervals are reported in parentheses.
*Immigrant fraction as calculated through the Bayesian method of Wilson and Rannala (2003).

Table 3. Analysis of variance (ANOVA) table testing for association between the explanatory variables origin, ecotype, year, and latitude with mean allelic richness of Oregon coastal coho salmon (*Oncorhynchus kisutch*) populations.

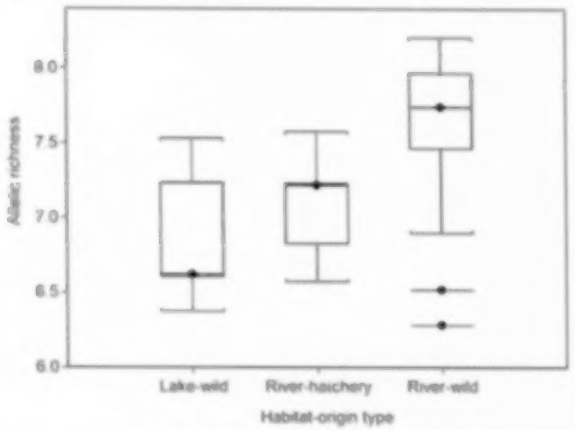
	df	Sum of squares	Mean squares	F	Pr(F)
Origin	1	0.9782	0.9782	4.4301	0.043
Ecotype	1	1.8633	1.8633	8.4384	0.007
Year	2	0.2716	0.1358	0.6150	0.547
Latitude	1	0.0045	0.0045	0.0203	0.888
Error	32	7.0661	0.2208		

Note: See text for variable descriptions.

year nor latitude appeared to be significant ($F_{[2,32]} = 0.615$, $p = 0.547$ and $F_{[1,32]} = 0.020$, $p = 0.888$, respectively Table 3). Given these results, we then collapsed the data into three categories: WildRiver, WildLake, and HatcheryRiver, recognizing that each of these population "types" could be making substantially different contributions to the overall mean of allelic richness. A Scheffé's multiple comparisons analysis indicated that mean allelic richness of wild lake rearing populations is significantly lower than wild river rearing populations (Fig. 3). One potential outlier, the Trask River population, presented markedly low mean allelic richness for a wild river population (Fig. 4). When this sample is removed, the Scheffé's multiple comparisons analysis suggests that wild river populations present significantly higher mean allelic richness than both wild lake and hatchery river origin coho.

Given the small sample sizes of both lake and hatchery populations and the disparate patterns of allelic richness observed between these groups and wild river coho salmon, linear regression analysis of mean allelic richness was conducted only for the latter. A latitudinal cline in neutral genetic diversity, peaking in the central region of the OCC ESU, can be observed by plotting mean allelic richness of wild river coho populations against the latitude at which they enter freshwater spawning grounds (Fig. 4). Initially, a model with a single linear term, latitude, was evaluated, but this variable alone was not significantly associated with variation in allelic richness ($F_{[1,26]} = 0.3509$, $p = 0.5587$). Given the curvature observed in the data (Fig. 4), a linear regression model containing a quadratic latitudinal term was tested and found to carry high significance, explaining 33.1% of

Fig. 3. Box plots depicting the range of mean allelic richness observed among coho (*Oncorhynchus kisutch*) populations from three habitat origin types: lake-wild, river-hatchery, river-wild. Boxes enclose the 25th through 75th quartile range, and whiskers are drawn to the nearest value not beyond the standard span from quartiles (i.e., 1.5 × interquartile range).



the variation observed in population mean allelic richness ($F_{[2,25]} = 6.184$, $p = 0.007$):

$$\mu[\text{Richness}|\text{Latitude}] = -501.93 + 23.02 \times \text{Latitude} - 0.26 \times \text{Latitude}^2$$

Moreover, when the potentially outlying Trask River sample was removed from this analysis, the model fit improved considerably, explaining 43% of the variation ($F_{[2,24]} = 9.048$, $p = 0.001$).

Discussion

Population structure and migration

The relatively weak genetic structure observed among coho salmon from Oregon coastal rivers suggests that migration among these populations has acted to reduce the effects of genetic drift and divergence. Tagging studies have suggested that during spawning migrations, coho may stray into non-natal streams more frequently than some other Pacific salmonids (Shapovalov and Taft 1954; Quinn 2005). Moreover, past human activities have served to mediate migration among Oregon coho populations through the practice

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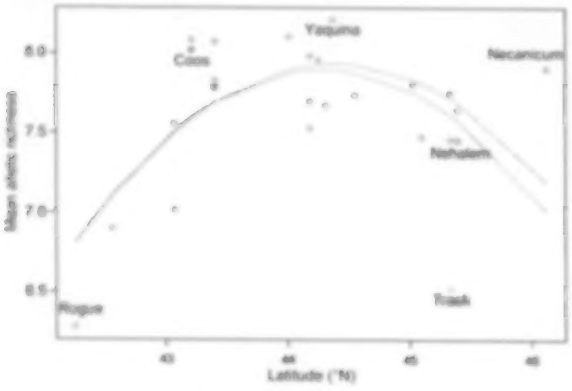
Fig. 4. Relationship between mean allelic richness of wild, river-rearing Oregon coastal coho salmon (*Oncorhynchus kisutch*) populations and the latitude at which they enter freshwater spawning grounds. The upper curve includes the Trask River sample point; the lower curve does not.



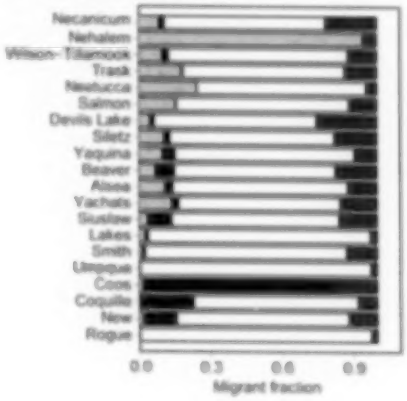
of hatchery stock transfers. Disentangling the relative influence of these two sources of migration is complicated by the shared ancestry of many hatchery and wild coho populations, as well as the unpredictable nature of genetic introgression from introduced hatchery stocks.

The immigrant fraction values we have estimated through the temporal F_{ST} method approximate those reported by Ford et al. (2004), although a direct comparison can only be made for the Smith River, which presents nearly identical values. Immigrant fractions were highest in the Yachats and Coquille populations, that in 2002 presented the lowest spawner abundance counts for the five populations examined (data not shown). Curiously, the temporal F_{ST} method provides a rather high m value for the large Nehalem population. It should be kept in mind, however, that several assumptions of the temporal F_{ST} method, namely HWE and symmetrical geneflow among populations, might not be met. By applying the Bayesian method of Wilson and Rannala (2003), we provide directional migration rate estimates that are not contingent on these assumptions.

Bayesian migration rate estimates tended to be higher than those of the temporal F_{ST} method and suggest that two of the larger populations function as migrant sources for numerous smaller populations (Fig. 5 and Appendix A, Table A2). Moreover, source–sink dynamics appear to be influenced by distance, as smaller basins appear to receive more migrants from the Coos in the south, whereas the Nehalem functions as the principal migrant source in the north (Fig. 5). Consequently, populations of the central coast, such as those from the Yaquina River, Alsea River, and Beaver Creek, receive relatively balanced immigrant contributions from the major source populations.

Two noteworthy sources of error may be compromising the accuracy of our Bayesian migration rate estimates. First, simulations performed by Wilson and Rannala (2003) indicate that their method lacks power when either few loci (less than 20) are employed or when population structure is weak. Given the nature of our data set, caution must therefore be taken when interpreting these results. The influence

Fig. 5. Oregon coho (*Oncorhynchus kisutch*) populations ordered from north (top) to south (bottom) as composed by individuals homing to their natal river (open) and migrants from the Nehalem (light gray), Coos (black), and all other sources combined (dark gray). Migrant contributions estimated with the program BAYESASS (Wilson and Rannala 2003).



of unmarked hatchery coho salmon may constitute a second source of error in our migration rate estimates. Most notably, in 1999, the Nehalem hatchery released 53 080 unmarked hatchery coho smolts. Given estimated smolt-to-adult survival rates of 4% to 8% (Logerwell et al. 2003), this hatchery cohort likely produced between 2123 and 4246 unmarked coho adults returning in 2002, a portion of which may have strayed into neighboring basins, thereby elevating the estimate of wild migrants from the Nehalem River. This event may also have generated major allele frequency changes within the Nehalem River population, affecting the Nehalem temporal F_{ST} estimate of m. In contrast, such confounding circumstances are not associated with the Coos source population, as only 139 unmarked Coos River hatchery smolts were released in 1999. Despite these factors, we believe that our results provide a useful index of migration rates and represent the first attempt at quantitatively describing source–sink dynamics among Oregon coho populations.

Dramatic census size increases, recorded for most coastal Oregon coho salmon populations during the sampling period of 1998–2002 (Fig. 6), have probable relevance to our findings. It is likely that the relatively high contemporary migration rates that we are reporting are the result of a spilling over effect, as the larger Coos and Nehalem populations responded more quickly to favorable conditions and provided large numbers of migrants to neighboring populations in 2002. Again, hatchery influence over coho population dynamics on the Nehalem River cannot be ignored and may have contributed to this population's rapid census size increase. Nevertheless, wild coho smolt production on the north fork of this river was estimated to be twice that of any other coastal Oregon site monitored by ODFW in 1999.

Regardless of the source, admixture resulting from migration would generate a transient signal of nonrandom mating. Such a scenario would serve to explain the major departures from HWE that we have detected in the majority of wild populations. Although recent population expansions may be

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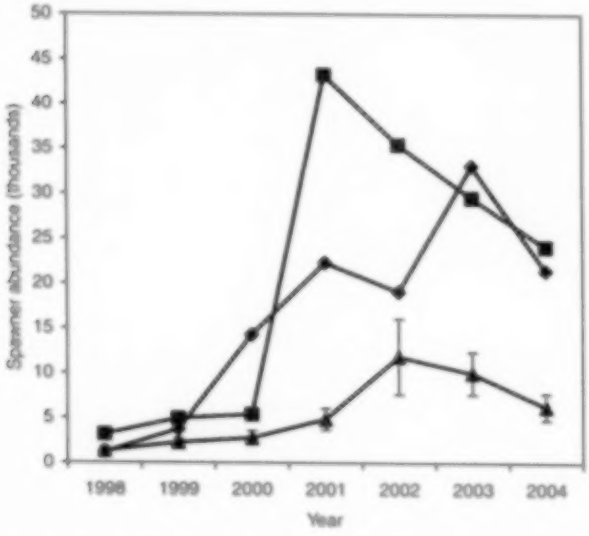
Fig. 6. Spawner abundance of coho salmon (*Oncorhynchus kisutch*) in the Nehalem (◆) and Coos (■) rivers, plotted together with the mean spawner abundance of 13 other Oregon coastal coho evolutionarily significant unit (OCC ESU) populations (▲: Necanicum, Tillamook, Nestucca, Salmon, Siletz, Yaquina, Alsea, Yachats, Siuslaw, Coquille, Tenmile Lake, Tahkenitch Lake, Siltcoos Lake) for the years 1998 through 2004. Error bars indicate standard error of mean estimate. Adapted from Oregon Department of Fisheries and Wildlife data (oregonstate.edu/Dept/ODFW/spawn/cohoabund.htm).



increasing this effect, it seems that high levels of geneflow among populations may be characteristic of Oregon coho salmon, as our $F_{ST}(\theta)$ estimates corroborate previously reported values for Oregon coho (Ford et al. 2004), yet are much lower than values reported for coho at more extreme locations of the species' North American distribution (Olsen et al. 2003; Bucklin et al. 2007).

The temporal and spatial scales over which natural populations form discrete units are seldom predictable and often dynamic. Whereas significant departures from HWE were observed when all individuals from a given basin were considered as a single population, most hatchery populations conformed to HWE expectations. Again, this observation suggests that considerable admixture or genetic substructure, not present in hatchery populations, exists within natural coho populations.

To evaluate the possibility of within-basin substructure, we conducted a hierarchical analysis of genetic covariance and tested for conformance with HWE expectations at the level of within-basin sampling sites for the Nehalem, Yachats, Smith, and Coos populations. Hierarchical analyses of genetic covariance provided only limited evidence for within-basin substructure, when contrasted with covariance levels associated with among-basin differences. However, among-site covariance did exceed among-year covariance; thus, it can be inferred that precocial males (jacks) mediate a higher level of geneflow among brood-year populations than occurs among spawning sites within the same basin. Concomitantly, nearly a third of all putative subpopulations did not conform to genotypic frequency expectations under HWE, even after sequential Bonferroni corrections. Thus, within-basin substructure appears to be both weak and more complex than a temporally stable system of spatially defined demographic units. Unfortunately, the use of carcass samples precluded our ability to detect for multiple, temporally structured populations within spawning years at sampling sites.

In all but one case, hatchery coho salmon populations appeared to be most similar to wild fish of the same river. This result is perhaps not surprising, as the hatchery populations examined were founded by local wild stock, with the exception of the Salmon River hatchery population. Moreover, the Coquille, Coos, and Cow Creek hatchery stocks receive regular supplementation from native wild fish. Although small sample sizes preclude statistical analyses, each of these three hatcheries presented higher allelic richness than both the Nehalem and Salmon hatchery populations, which do not incorporate wild stock into their breeding programs. Overall, the general pattern suggests that hatchery populations possess significantly lower levels of allelic richness than wild river populations.

Allelic richness

Founder effects, bottlenecks, and inbreeding may all contribute to the relatively low allelic richness observed in Oregon hatchery coho salmon populations. However, these processes may also act over natural populations. Coho salmon from Oregon rivers have been shown to be more diverse than more northerly populations with both allozymes (Wehrhahn and Powell 1987) and mtDNA sequence data (Smith et al. 2001). These findings have been interpreted as a signal of bottlenecks and founder effects from a limited number of post-Pleistocene colonizations by coho from Oregon and California refugia. Interestingly, the coastal lakes flanking the mouth of the Umpqua River are also believed to be of late Pleistocene origin (Cooper 1958; Johnson et al. 1985), far younger than most rivers along the Oregon coast. These basins therefore became available for colonization by coho during the same period that more northerly, glaciated rivers of Washington, British Columbia, and Alaska became inhabitable. Founder effects may then also serve to explain the relatively low levels of allelic richness observed in coho populations from Oregon coastal lakes, as compared with neighboring populations.

The highest levels of allelic richness were found in river populations from the central region of the OCC ESU. Interestingly, it appears that coho salmon populations of this region receive the most balanced contributions from the two primary, geographically distant migrant sources of the ESU: the Nehalem and Coos river populations (Fig. 5).

Only one population from another ESU was included in our analyses, that of the Rogue River in southern Oregon, and it presented the lowest level of allelic richness observed among our samples. While sampling across additional ESUs could certainly broaden the scope of our findings, the intensive within-ESU sampling strategy we have employed has provided sufficient resolution to detect both continuous and categorical patterns of allelic richness that might otherwise have been overlooked or misinterpreted.

In conclusion, our findings suggest that both natural and

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anthropogenic forces have served to shape the contemporary patterns of coho salmon genetic diversity along the Oregon coast. Relatively high migration rates from a limited number of large, geographically distant source populations appear to modulate spatial genetic structure while maintaining elevated levels of allelic richness within the core of the OCC ESU. In concert with local adaptations, the source–sink dynamics we have described may very well represent a long-standing ecological mechanism responsible for generating and maintaining genetic diversity in Oregon coastal coho salmon populations.

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Appendix A

Tables A1 and A2 appear on the following pages.

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Table A1. Pairwise θ values for Oregon coastal coho salmon (*Oncorhynchus kisutch*) populations as defined by basins (data above diag-

	Umpqua	Yaquina	Yachats	Wilson	Trask	Tillamook	Tenmile	Tahkenitch	Sutton	Smith	Siuslaw	Siltcoos
Alsea	0.010	0.014	0.015	0.020	0.021	0.025	0.031	0.031	0.032	0.015	0.006	0.032
Beaver	0.015	0.011	0.016	0.022	0.014	0.025	0.043	0.028	0.039	0.026	0.007	0.032
Coos	0.013	0.013	0.025	0.037	0.044	0.032	0.018	0.020	0.027	0.010	0.011	0.021
Coquille	0.026	0.025	0.040	0.047	0.066	0.038	0.023	0.031	0.035	0.022	0.021	0.028
Devil's	0.035	0.022	0.045	0.021	0.029	0.023	0.064	0.047	0.046	0.045	0.023	0.035
Necanicum	0.013	0.008	0.008	0.016	0.019	0.009*	0.030	0.031	0.041	0.019	0.011	0.032
Nehalem	0.020	0.015	0.014	0.011	0.006*	0.024	0.051	0.039	0.042	0.028	0.012	0.037
Nestucca	0.016	0.014	0.008*	0.016	0.004*	0.030	0.036	0.034	0.034	0.024	0.010	0.032
New	0.026	0.024	0.041	0.053	0.061	0.062	0.028	0.028	0.027	0.028	0.024	0.024
Salmon	0.022	0.015	0.024	0.010	0.002*	0.017	0.054	0.044	0.049	0.035	0.015	0.040
Siletz	0.015	0.016	0.019	0.018	0.013	0.022	0.047	0.040	0.040	0.028	0.013	0.028
Siltcoos	0.028	0.021	0.044	0.032	0.053	0.055	0.017	0.007*	0.005*	0.030	0.018	—
Siuslaw	0.007	0.006	0.011	0.015	0.020	0.020	0.026	0.015	0.021	0.011	—	
Smith	0.007	0.022	0.019	0.039	0.043	0.032	0.021	0.028	0.033	—		
Sutton	0.028	0.029	0.047	0.043	0.064	0.059	0.019	0.009	—			
Tahkenitch	0.025	0.024	0.037	0.041	0.056	0.056	0.019	—				
Tenmile	0.026	0.034	0.029	0.058	0.068	0.062	—					
Tillamook	0.024	0.019	0.041	0.020	0.031	—						
Trask	0.025	0.020	0.019	0.015	—							
Wilson	0.027	0.016	0.027	—								
Yachats	0.015	0.020	—									
Yaquina	0.016	—										
Umpqua	—											

*Not significantly different from zero at $p < 0.05$ with 1000 permutations.

Table A2. Directional migration rates among coho (*Oncorhynchus kisutch*) populations of the Oregon coast, as estimated by the program

Source	Necanicum	Nehalem	Wilson–Tillamook	Trask	Nestucca	Salmon	Devil's	Siletz	Yaquina	Beaver
Necanicum	0.678	0.000	0.002	0.003	0.001	0.002	0.002	0.002	0.001	0.003
Nehalem	0.086	0.945	0.098	0.179	0.243	0.154	0.047	0.106	0.100	0.069
Wilson–Tillamook	0.010	0.025	0.754	0.017	0.006	0.011	0.159	0.007	0.009	0.009
Trask	0.003	0.001	0.002	0.679	0.001	0.002	0.003	0.002	0.001	0.003
Nestucca	0.005	0.001	0.003	0.007	0.709	0.003	0.004	0.006	0.004	0.013
Salmon	0.003	0.007	0.007	0.005	0.004	0.723	0.005	0.010	0.003	0.003
Devil's	0.003	0.001	0.003	0.004	0.002	0.002	0.683	0.003	0.002	0.003
Siletz	0.004	0.001	0.005	0.006	0.002	0.002	0.004	0.691	0.002	0.005
Yaquina	0.005	0.003	0.011	0.022	0.006	0.005	0.009	0.014	0.753	0.037
Beaver	0.004	0.000	0.003	0.004	0.001	0.002	0.003	0.002	0.001	0.676
Alsea	0.020	0.005	0.010	0.016	0.003	0.039	0.010	0.025	0.028	0.008
Yachats	0.004	0.001	0.002	0.004	0.001	0.002	0.003	0.002	0.001	0.003
Siuslaw	0.006	0.001	0.012	0.004	0.002	0.002	0.006	0.009	0.010	0.012
Lakes – lower Umpqua	0.019	0.002	0.007	0.006	0.004	0.005	0.016	0.016	0.015	0.030
Smith	0.018	0.001	0.008	0.005	0.002	0.008	0.005	0.006	0.003	0.004
Umpqua	0.104	0.002	0.039	0.021	0.005	0.024	0.015	0.009	0.004	0.033
Coos	0.021	0.003	0.027	0.008	0.003	0.006	0.017	0.025	0.053	0.081
Coquille	0.003	0.001	0.003	0.005	0.002	0.005	0.003	0.003	0.001	0.004
New	0.004	0.001	0.003	0.005	0.002	0.002	0.006	0.003	0.007	0.005
Rogue	—	0.001	0.003	0.004	0.002	0.003	0.003	0.004	0.002	0.004

Note: First column (Source) lists source of migrants (e.g., migration rate from Alsea to Beaver = 0.008).

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onal) and tributaries of the Umpqua River (lower right inset).

Siletz	Salmon	New	Nestucca	Nehalem	Necanicum	Devil's	Coquille	Coos	Beaver	Alsea
0.013	0.014	0.034	0.008	0.016	0.012	0.030	0.030	0.018	0.013	—
0.009	0.014	0.033	0.008	0.014	0.014	0.026	0.041	0.025	—	
0.029	0.034	0.022	0.027	0.027	0.019	0.042	0.011	—		
0.043	0.048	0.022	0.041	0.049	0.033	0.058	—			
0.019	0.021	0.050	0.027	0.021	0.025	—				
0.010	0.008	0.038	0.010	0.013	—					
0.015	0.013	0.046	0.008	—						
0.009	0.008	0.038	—							
0.039	0.052	—								
0.009	—									
—										

Inset: Smith	South Umpqua	Calapooya	EB	Main Umpqua	Cow Creek hatchery	
					0.004	Main Umpqua
				0.007	0.004	EB
			0.008	0.012	0.020	Calapooya
		0.004	0.009	0.013	0.017	South Umpqua
	0.020	0.010	0.010	0.015	0.016	Smith
0.012	0.030	0.024	0.014	0.025	0.027	Lower Umpqua

BAYESASS (Wilson and Rannala 2003).

Alsea	Yachats	Siuslaw	Lakes – lower Umpqua	Smith	Umpqua	Coos	Coquille	New	Rogue
0.002	0.004	0.001	0.000	0.001	0.000	0.001	0.002	0.002	—
0.108	0.138	0.036	0.024	0.013	0.002	0.016	0.003	0.017	0.002
0.007	0.009	0.015	0.002	0.003	0.001	0.008	0.004	0.006	0.002
0.002	0.004	0.002	0.001	0.001	0.000	0.001	0.002	0.002	0.001
0.005	0.020	0.001	0.001	0.002	0.001	0.002	0.004	0.005	0.001
0.003	0.004	0.004	0.001	0.001	0.001	0.001	0.004	0.002	0.001
0.002	0.004	0.001	0.001	0.001	0.000	0.001	0.002	0.002	0.001
0.004	0.005	0.002	0.001	0.002	0.001	0.001	0.002	0.004	0.001
0.006	0.009	0.032	0.002	0.010	0.001	0.009	0.004	0.005	0.002
0.002	0.004	0.001	0.001	0.001	0.000	0.001	0.002	0.002	0.001
0.735	0.005	0.007	0.002	0.002	0.003	0.026	0.006	0.004	0.002
0.002	0.680	0.001	0.001	0.001	0.000	0.001	0.002	0.002	0.001
0.002	0.008	0.705	0.001	0.004	0.002	0.002	0.007	0.004	0.001
0.033	0.033	0.048	0.933	0.037	0.002	0.103	0.018	0.047	0.002
0.009	0.007	0.002	0.008	0.847	0.011	0.014	0.008	0.004	0.002
0.040	0.021	0.034	0.007	0.059	0.970	0.002	0.010	0.022	0.003
0.034	0.029	0.102	0.014	0.013	0.003	0.809	0.226	0.140	0.002
0.002	0.010	0.002	0.001	0.001	0.001	0.002	0.691	0.005	0.001
0.002	0.008	0.002	0.001	0.001	0.001	0.001	0.003	0.725	0.002
0.004	0.005	0.002	0.000	0.002	0.000	0.001	0.005	0.004	0.971

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The effects of barotrauma on the catch-and-release survival of southern California nearshore and shelf rockfish (Scorpaenidae, *Sebastes* spp.)

Erica T. Jarvis and Christopher G. Lowe

Abstract: Two experiments were used to assess the effects of barotrauma on initial capture survival and short-term post-recompression survival of line-caught (range 18–225 m) southern California rockfish (*Sebastes* spp.). Occurrence of external and internal signs of barotrauma was characterized across all species. Despite species-specific differences in the extent of barotrauma observed, initial capture survival of rockfish held in a live well for a 10-min period following capture was 68% overall (19 species, $n = 168$). Overall 2-day survival of rockfish following recompression in cages was also 68% (17 species, $n = 257$). Short-term survival varied across species (range 36% to 82%), as did the occurrence of external signs of barotrauma. The degree of external signs of barotrauma was not a significant predictor of initial capture survival or short-term survival. The most significant predictor of short-term survival was surface holding time, with short-term survival increasing with decreasing surface holding time. These results suggest that rapid recompression of rockfish can significantly decrease discard mortality and could potentially enhance rockfish conservation.

Résumé : Deux expériences nous ont servi évaluer les effets du barotraumatisme sur la survie initiale à la capture et la survie à court terme après la recompression de sébastes (*Sebastes* spp.) du sud de la Californie capturés à la ligne (étendue des profondeurs de 18–225 m). Nous avons observé des signes externes et internes de barotraumatisme chez toutes les espèces. Malgré des différences spécifiques de l'importance du barotraumatisme, la survie initiale à la capture des sébastes gardés dans un vivier pendant 10 min suivant la capture est globalement de 68 % (19 espèces, $n = 168$). La survie globale des sébastes gardés dans des cages pendant 2 jours après la recompression est aussi de 68 % (17 espèces, $n = 257$). La survie à court terme varie d'une espèce à l'autre (étendue de 36 à 82 %), de même que la présence de signes externes de barotraumatisme. L'importance des signes externes de barotraumatisme ne permet pas de prédire avec assurance la survie initiale à la capture, ni la survie à court terme. La variable la plus significative pour prédire la survie à court terme est la durée de la retenue du poisson en surface, la survie augmentant en fonction inverse de la durée de la retenue en surface. Ces résultats indiquent qu'une recompression rapide des sébastes peut réduire de façon significative la mortalité lors de leur rejet à la mer et pourrait potentiellement favoriser la conservation des sébastes.

[Traduit par la Rédaction]

Introduction

Rockfish of the genus *Sebastes* are an extremely diverse group, inhabiting a variety of depths and habitats worldwide (Love et al. 2002). The majority of these species occur in the North Pacific, and historically, many have comprised major commercial and recreational fisheries. In southern California, rockfish catches have been an important component of the winter recreational fishery since the 1950s (Dotson and Charter 1998); however, there is strong evidence indicating that many rockfish populations have suffered from excessive harvest impacts over the last two decades (Love et al. 1998; Mason 1998; Butler et al. 2003). Growing concern regarding bycatch (i.e., incidentals and discards) mortality in the recreational rockfish fishery has prompted research into rockfish physiology and the effects of angling-induced barotrauma on their survival (Parker et al. 2006; Hannah and Matteson 2007). Upon rapid decompression, rockfish, which are physoclistic, suffer internal injury and positive buoyancy as a result of overexpansion of gases within the swim bladder and other highly vascularized tissues. Consequently, many discarded rockfish are unable to swim down deep enough to force recompression of gases within their bodies. Discard mortality in the recreational fishery is assumed to be high, as floating fish at the surface eventually succumb to thermal shock and (or) bird or marine mammal predation.

Recent research on Northeast Pacific coast rockfish suggests that assisted release of discards may increase postrelease survival, as external signs of barotrauma were alleviated following recompression of fish to depth (Hannah and Matteson 2007). However, several studies on other

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physoclistic species have found no correlation between degree of barotrauma and survival (Gitschlag and Renaud 1994; Rummer and Bennett 2005). External signs of barotrauma documented for a few rockfish species include stomach eversion, exophthalmia (bulging eyes), corneal gas bubbles, and subcutaneous gas bubbles (Gotshall 1964; Lea et al. 1999; Hannah and Matteson 2007). Internal signs of barotrauma have not been characterized for rockfish, but in other physoclists, these signs have included arterial embolism, swim bladder rupture, hematoma, hemorrhage, and organ torsion (Gitschlag and Renaud 1994; Rummer and Bennett 2005). The effects of internal barotrauma on immediate and delayed mortality in the field have not yet been reported, and the condition and longer-term survival of line-caught rockfish recompressed in the field is still largely unknown.

Releasing rockfish at depth aims to decrease the probability of discard mortality, but the practice can only be effective if evidence indicates that a high enough percentage of fish returned to depth recover and survive. The primary objectives of this study were (*i*) to characterize external and internal signs of angling-induced barotrauma in rockfish and (*ii*) to quantify initial survival (within 10 min of capture) and short-term (2-day) survival following recompression in cages. An understanding of postrecompression survival in rockfish will enable fisheries managers to assess the utility of catch-and-release regulations, not only in southern California, but also along the Northeast Pacific coast, and offer resource managers more accurate population parameters for stock assessments.

Materials and methods

Characterization of barotrauma and initial capture survival

Nearshore and shelf rockfish were captured by hook-and-line from the Palos Verdes peninsula (depth 30–70 m), Southeast Bank off Huntington Beach (50–90 m), at the offshore petroleum platform Gilda off the Ventura coast (55 m), and off Santa Catalina Island (18–96 m) between October 2004 and March 2006 (Fig. 1). Fishing was designed to target demersal species; all fishing lines were fished on the sea floor and arranged with one to two dropper loops tied approximately 22 cm above an 8–12 oz torpedo weight. We recorded bottom depth and the time of day that each fish was landed. Each fish was measured (standard length) and the presence of external signs of barotrauma including stomach eversion (SE), exophthalmia (EX), corneal gas bubbles (CB), subcutaneous gas bubbles (SB), and prolapsed cloaca (PC) were recorded. Handling time to measure and examine fish was kept to less than 2 min. Fish were placed in a live well or cooler with fresh seawater for 10 min, after which each fish was assessed for signs of gill ventilation as an indicator of initial capture survival. After noting survival, fish were euthanized and dissected within 24–48 h after capture. Internal signs of barotrauma were recorded as presence of visible tears in the tunica internus of the swim bladder (ST), organ torsion (OT), hemorrhage (HE), and presence and location of arterial gas embolisms in the pericardial chamber and at the swim bladder gas gland and oval (AE). Subcutaneous gas bubbles and arterial embolism were not initially documented at the onset of this experiment, thus sample sizes for these signs vary.

Analysis

Percent occurrence and percent survival (±95% binary confidence intervals) were reported for each species and for all species combined (adjusted Wald method; Sauro and Lewis 2005). We used a general linear model (GLM, STATISTICA 7.1; StatSoft, Inc., Tulsa, Oklahoma) to examine the relationship between depth of capture and the total number of external and internal signs of barotrauma, including species in the model as a possible effect. Barotrauma effects were assessed using a Fisher exact test, comparing the proportion of survivors with and without a given sign of barotrauma. Binary logistic regression (MINITAB release 14.20; Minitab Inc., State College, Pennsylvania) was used to model the extent of barotrauma as a predictor of initial survival (barotrauma model I, external signs; barotrauma model II, internal signs). The extent of barotrauma or barotrauma category was defined by specific combinations of different signs of barotrauma observed in captured fish and was treated as a single, categorical, independent variable. The models were limited to those specific combinations of barotrauma that occurred in at least 10 fish. Additionally, survival of fish showing each specific combination of barotrauma was reported relative to fish showing no signs of barotrauma. In these models, we included only individuals for which all signs were accounted.

Characterization of barotrauma and 2-day postrecompression survival

During the summers of 2004 and 2005, nearshore and shelf rockfish were captured by hook-and-line aboard the R/V *Yellowfin* near Southeast Bank (50–89 m; Fig. 1). For each fish, we recorded bottom depth, time of capture, standard length, and the presence of external signs of barotrauma. Each fish was externally tagged for individual identification with a 4 cm long dart tag (Hallprint) and placed in a live well prior to release. Fish were then transferred to polyvinyl chloride (PVC) coated wire mesh cages (4 cm × 4 cm mesh, 1.2 m × 1.2 m × 1.5 m). Up to six cages were deployed during each trip, with as many as 12 fish per cage. Each cage was lowered to the original fish capture depth and left for 2 days on soft substratum adjacent to fished reefs. To account for differences in total surface holding duration among individuals, we recorded the exact time of each cage deployment. Two cages per trip were deployed with StowAway TidbiT temperature data loggers (Onset Computer Corp., Bourne, Mass.) to record seafloor water temperatures. Differences in water temperature between the sea floor and sea surface (i.e., temperature differential) were recorded for each trip to account for temperature change experienced by rockfish upon capture.

Two days following fish collection and recompression, each cage was pulled up to a depth of 20 m where a team of divers met the cage and assessed each fish for mortality and external signs of barotrauma. As the greatest change in pressure and, hence, gas expansion occurs within the first

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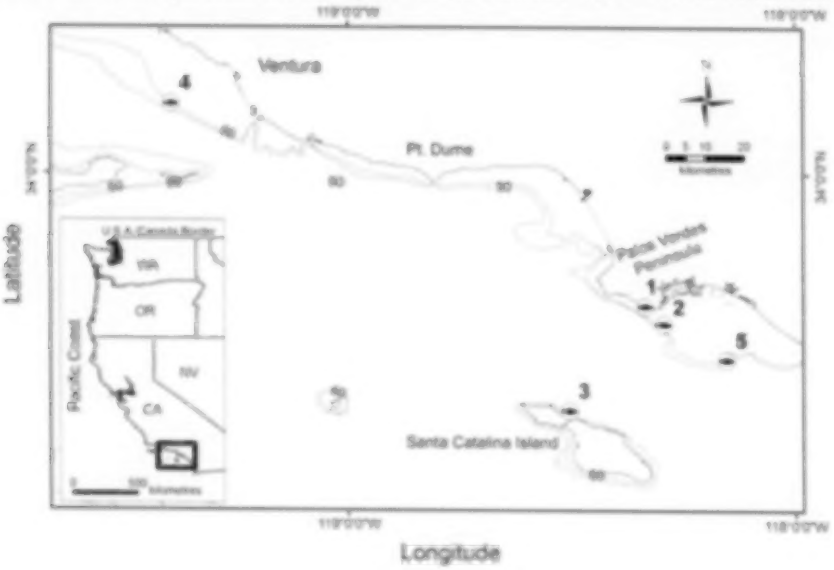
Fig. 1. Fishing locations of nearshore and shelf rockfish captured by hook-and-line in southern California from depths of 18–220 m (October 2004 to August 2006): (1) White's Point outfall pipe, (2) Rockpile, (3) Ship Rock, (4) platform Gilda, and (5) Southeast Bank. Shaded lines represent 60 m and 90 m depth contours. Inset depicts southern California in relation to the Pacific coast of the United States.



10 m of the surface, this observation depth of 20 m was chosen to greatly reduce the probability of barotrauma injury resulting from the second decompression event. All dead fish and a subset of live fish per cage were saved for examination of internal barotrauma injury as described above; all remaining live fish were released.

Longer-term survival

In addition to a unique tag number, all external tags used in the cage experiments contained a phone number for reporting recaptured rockfish. To record evidence of longer-term survival, all tag returns were logged by tag identification number, species, date of recapture, location of recapture, and fisher contact.

Analysis

Chi-square tests of independence were used to test for species-specific differences in both the percent occurrence of each sign of barotrauma and percent survival. Fisher exact tests and logistic regression were again used to test for barotrauma effects as described in the initial capture study. An additional logistic regression model (PROC LOGISTIC, SAS 9.1.3; SAS Institute Inc., Cary, North Carolina) tested the effects of depth, species, fish length, cage density, temperature differential, and surface holding duration on survival (overall model). For this model, we included only data for species with a sample size greater than 15. A reduced model was then designed using only significant variables from the overall model. Because several variables, including surface holding duration and temperature differential, could not be controlled in the field, we did not include interactions. To account for differences in species survival due to different temperature differentials or surface holding times, an analysis of variance (ANOVA) was used to compare temperature differential experienced by each species upon capture, and Kruskal–Wallis was used to test for differences in median surface times experienced by each species prior to recompression.

Results

Barotrauma and initial capture survival

One-hundred and sixty-eight rockfish representing 21 species were captured over a 1.5-year period from depths of 18–96 m; vermilion rockfish, *Sebastes miniatus*; greenspotted rockfish, *Sebastes chlorostictus*; olive rockfish, (*Sebastes serranoides*); halfbanded rockfish (*Sebastes semicinctus*), rosy rockfish (*Sebastes rosaceous*), and honeycomb rockfish (*Sebastes umbrosus*) comprised the majority of the catch (Table 1). Initial capture survival of rockfish was 68% overall (95% confidence interval, CI: 60% to 75%).

The most common external signs of barotrauma observed in the overall catch ($n = 168$) were subcutaneous gas bubbles (SB, 76% of $n = 157$), stomach eversion (SE, 63%), and exophthalmia (EX, 52%); the most common signs of internal barotrauma were arterial embolism (AE, 68% of $n = 144$), hemorrhage (HE, 64%), and organ torsion (OT, 33%). The percent occurrence of each barotrauma type and the number of frequently occurring signs of barotrauma were species-specific (Table 2). Stomach eversion occurred frequently among brown rockfish (*Sebastes auriculatus*), flag rockfish (*Sebastes rubrivinctus*), greenspotted, halfbanded, honeycomb, and rosy rockfish, starry rockfish (*Sebastes constellatus*), treefish (*Sebastes serriceps*), and widow rockfish (*Sebastes ensifer*), and swim bladder tears (ST) occurred frequently (82%) only in olive rockfish. For the catch as a whole, there was a significant, yet weak, positive correlation between the number of external and internal signs of barotrauma observed in each fish ($r = 0.28$,

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Table 1. Sample size (n), catch (%), and depth (m) of capture of nearshore and shelf rockfish captured by hook-and-line in southern California (initial capture survival experiment, October 2004 to March 2006).

Common name	Scientific name	n	Catch (%)	Depth (m)
Vermilion rockfish	*Sebastes miniatus*	35	21	35–96
Greenspotted rockfish	*Sebastes chlorostictus*	19	11	75–189
Olive rockfish	*Sebastes serranoides*	16	10	23–53
Halfbanded rockfish	*Sebastes semicinctus*	15	9	53–64
Rosy rockfish	*Sebastes rosaceus*	12	7	54–152
Honeycomb rockfish	*Sebastes umbrosus*	12	7	46–76
Widow rockfish	*Sebastes entomelas*	9	5	53
Flag rockfish	*Sebastes rubrivinctus*	9	5	55–60
Copper rockfish	*Sebastes caurinus*	7	4	53–69
Calico rockfish	*Sebastes dalli*	7	4	53
Treefish	*Sebastes serriceps*	5	3	18–53
Brown rockfish	*Sebastes auriculatus*	4	2	24–53
Starry rockfish	*Sebastes constellatus*	4	2	46–68
Chilipepper	*Sebastes goodei*	3	2	140
Greenstriped rockfish	*Sebastes elongatus*	2	1	177
Squarespot rockfish	*Sebastes hopkinsi*	2	1	61–69
Freckled rockfish	*Sebastes lentiginosus*	2	1	61–76
Bocaccio	*Sebastes paucispinis*	2	1	76–79
Kelp rockfish	*Sebastes atrovirens*	1	1	30
Yellowtail rockfish	*Sebastes flavidus*	1	1	53
Canary rockfish	*Sebastes pinniger*	1	1	53
Total		168	100	

Table 2. Percent occurrence of barotrauma in nearshore and shelf rockfish (only species with $n > 3$) following hook-and-line capture from depths of 18–189 m in southern California (initial capture survival experiment, October 2004 to March 2006).

		Occurrence of barotrauma (%)							
		External signs				Internal signs			
Common name	n	SE	EX	CB	PC	ST	HE	OT	No. of traumas > 50%
Olive rockfish	16	19	6	6	0	*88*	13	0	1
Treefish	5	*60*	40	20	0	20	*80*	0	2
Halfbanded rockfish	15	67	13	0	13	27	*60*	*53*	3
Widow rockfish	9	*100*	0	0	11	33	33	*100*	2
Brown rockfish	4	75	25	0	25	0	25	0	1
Calico rockfish	7	43	29	14	43	14	29	43	0
Copper rockfish	7	29	*86*	*71*	14	0	43	29	2
Flag rockfish	9	*100*	33	22	11	0	*100*	*78*	3
Starry rockfish	4	*100*	*100*	25	0	0	*100*	0	3
Honeycomb rockfish	12	92	42	25	8	0	67	17	2
Vermilion rockfish	35	40	*71*	*57*	14	6	*74*	26	3
Rosy rockfish	12	*50*	*83*	67	0	0	*75*	*50*	5
Greenspotted rockfish	19	*84*	*89*	*79*	11	6	*89*	32	4

Note: Species are arranged in order of most frequent capture depth. Values in italics indicate signs of barotrauma occurring in at least 50% of individuals. SE, stomach eversion; EX, exophthalmia; CB, corneal gas bubbles; SE, stomach eversion; PC, prolapsed cloaca; ST, swim bladder tear; HE, hemorrhage; OT, organ torsion.

$p < 0.01$). The degree of barotrauma was greatest for the two species caught deepest, rosy and greenspotted rockfish. However, regardless of species, depth had a significant positive relationship with the total number of traumas observed in rockfish individuals (GLM, $n = 168$, $R^2 = 0.35$, $F = 3.61$, $p < 0.001$).

Initial capture survival varied by species (Fig. 2). Eight of 12 species (with $n > 3$) had greater than 75% initial capture survival, whereas olive and rosy rockfish had low survival. Although fish caught at deeper depths generally showed higher numbers of trauma, species caught at shallow depths showed relatively similar survival proportions as species caught in deeper depths.

Overall percent survival was significantly higher for fish

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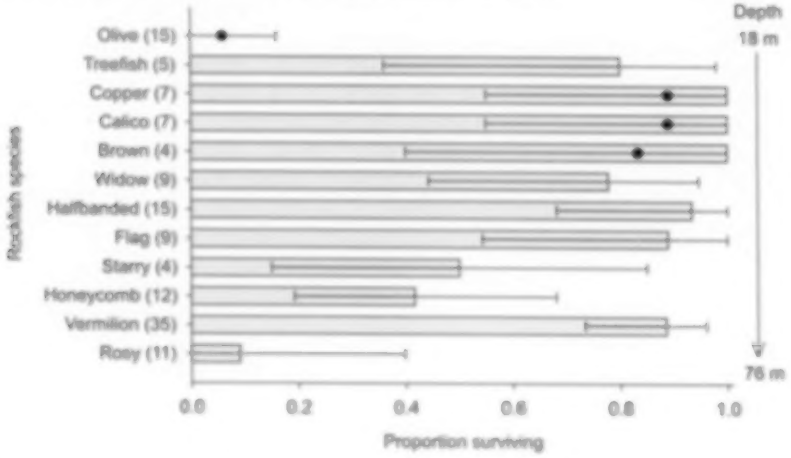
Fig. 2. Initial capture survival (±95% binary confidence intervals) of southern California nearshore and shelf rockfish (only species with $n > 3$) captured by hook-and-line from depths of 18–96 m after a 10-min holding period on deck in a live well (initial capture survival experiment, October 2004 to March 2006). Species are arranged in order of capture depth. Numbers in parentheses indicate sample sizes. Solid circles represent LaPlace Point estimates for species with survival of either 0% or 100%.



without arterial embolism (85%) than for fish with arterial embolism (58%; two-sided Fisher exact test, $p = 0.002$). Percent survival was also significantly higher for fish without swim bladder tears (73%) than for fish with swim bladder tears (42%; two-sided Fisher exact test, $p = 0.002$). Fifty-four percent of dead fish showing arterial embolism were rosy and olive rockfish, and 87% of dead fish showing swim bladder tears were olive rockfish. Externally, rockfish commonly showed five different categories of barotrauma: (*i*) the absence of all signs of barotrauma, (*ii*) the presence of only EX and CB, (*iii*) only SE, (*iv*) only SE and EX, or (*v*) all three signs together (SE, EX, and CB). Internally, rockfish commonly showed eight different categories of barotrauma: (*i*) the absence of all signs of barotrauma, (*ii*) the presence of only AE, (*iii*) only HE, (*iv*) only AE and HE, (*v*) only AE and OT, (*vi*) only AE and ST, (*vii*) only HE and OT, and (*viii*) HE, OT, and AE together. The effect of external or internal barotrauma category on initial survival was not found to be statistically significant (barotrauma model I, external signs, logistic regression, $\chi^2 = 8.82$, df = 4, $p = 0.07$; barotrauma model II, internal signs, logistic regression, $\chi^2 = 13.71$, df = 7, $p = 0.06$). Within barotrauma model II, fish showing both arterial embolism and swim bladder tears had significantly lower survival than fish showing no signs of barotrauma ($p = 0.02$, OR = 0.05); however, olive rockfish was the only species showing both AE and ST.

Barotrauma and 2-day postrecompression survival

We captured 344 rockfish comprising 17 species over seven trips. Of these, 328 fish were assessed for external signs of barotrauma, and 257 fish were recompressed to original capture depth in 42 cage deployments. A minimum of three fish and a maximum of 12 were recompressed in a single cage; the average number of fish per cage (i.e., cage density) was seven. The average capture depth was 71 m and ranged from 55 m to 81 m. Five species, vermilion rockfish, bocaccio (*Sebastes paucispinis*), and flag, squarespot (*Sebastes hopkinsi*), and honeycomb rockfish, comprised the majority (82%) of the catch (Table 3). Overall short-term survival of rockfish was 68% (95% CI: 62% to 73%). Although external signs of barotrauma were common upon capture, virtually none of the caged fish (<1%) showed external signs of barotrauma 2 days after recompression.

Of 328 fish, the most common external signs of barotrauma observed upon capture were stomach eversion (SE, 88%), exophthalmia (EX, 47%), and corneal gas bubbles (CB, 36%). The occurrence of prolapsed cloaca was not common (PC, 7%). Two days after recompression, the majority of fish were observed with cloudy corneas. Initially, the cloudy corneas were assumed to be a residual effect of ocular trauma as a result of the initial capture event, but 75% of fish having no signs of ocular trauma upon capture also showed cloudy corneas.

The extent of external signs of barotrauma observed upon capture was species-specific (Table 4). Flag, starry, and vermilion rockfish each frequently showed three signs of barotrauma. In contrast, only one sign of barotrauma, stomach eversion, frequently occurred in bocaccio and halfbanded, speckled (*Sebastes ovalis*), and squarespot rockfish. The occurrence of exophthalmia and corneal gas bubbles significantly differed among the five most abundant species (exophthalmia, $\chi^2 = 44.61$, df = 4, $p \leq 0.001$; corneal gas bubbles, $\chi^2 = 24.86$, df = 4, $p \leq 0.001$); however, there was no significant difference in the occurrence of stomach eversion among these species ($\chi^2 = 3.05$, df = 4, $p > 0.05$).

Overall short-term survival (68%) was similar to that found in the initial capture survival experiment (also 68%). Two-day postrecompression survival was species-specific and ranged from 36% (95% CI: 21% to 56%) for squarespot rockfish ($n = 27$) to 82% (95% CI: 51% to 96%) for starry rockfish ($n = 11$). There was a significant difference in species survival among the five most abundant species ($\chi^2 = 21.6$, df = 5, $p \leq 0.0001$) (Fig. 3); squarespot rockfish showed the lowest survival, whereas bocaccio showed the highest survival (95% CI: 79 to 89%). By species, there

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Table 3. Sample size (n), catch (%), and depth (m) of capture of nearshore and shelf rockfish captured by hook-and-line in southern California (2-day postrecompression survival experiment, summer 2005 and 2006).

Common name	Scientific name	n	Catch (%)	Depth (m)
Vermilion rockfish	*Sebastes miniatus*	73	28.5	55–86
Bocaccio	*Sebastes paucispinis*	64	25.0	57–89
Flag rockfish	*Sebastes rubrivinctus*	29	11.3	55–89
Squarespot rockfish	*Sebastes hopkinsi*	28	10.9	55–83
Honeycomb rockfish	*Sebastes umbrosus*	17	6.6	56–84
Starry rockfish	*Sebastes constellatus*	11	4.3	58–89
Speckled rockfish	*Sebastes ovalis*	11	4.3	80–84
Chilipepper	*Sebastes goodei*	7	2.7	57–84
Halfbanded rockfish	*Sebastes semicinctus*	5	1.6	59–84
Greenspotted rockfish	*Sebastes chlorostictus*	3	1.2	80–83
Olive rockfish	*Sebastes serranoides*	2	0.8	56, 83
Copper rockfish	*Sebastes caurinus*	2	0.8	56, 84
Yellowtail rockfish	*Sebastes flavidus*	1	0.4	84
Rosy rockfish	*Sebastes rosaceous*	1	0.4	82
Greenstriped rockfish	*Sebastes elongatus*	1	0.4	84
Freckled rockfish	*Sebastes lentiginosus*	1	0.4	82
Canary rockfish	*Sebastes pinniger*	1	0.4	89
Total		257	100.0	

Table 4. Percent occurrence of barotrauma in nearshore and shelf rockfish (only species with $n > 10$) following hook-and-line capture from depths of 55–89 m in southern California (2-day postrecompression survival experiment, summer 2005 and 2006).

		Occurrence of barotrauma (%)								
		External signs					Internal signs[a]			
Common name	n	EX	CB	SE	PC	n	ST	HE	OT	AE
Bocaccio	66	33	29	92	0	12	0	*100*	33	25
Flag rockfish	32	*63*	*59*	*84*	13	5	0	*80*	0	40
Halfbanded rockfish	41	12	7	*93*	12	0	—	—	—	—
Honeycomb rockfish	28	*57*	46	*82*	4	2	—	—	—	—
Speckled rockfish	13	8	15	*85*	23	0	—	—	—	—
Squarespot rockfish	40	25	15	*88*	15	7	43	*100*	29	29
Starry rockfish	11	*82*	*64*	*91*	0	4	25	*100*	0	*50*
Vermilion rockfish	75	*81*	*52*	*92*	0	13	8	*85*	15	*69*

Note: Values in italics indicate signs of barotrauma occurring in at least 50% of individuals. EX, exophthalmia; CB, corneal gas bubbles; SE, stomach eversion; PC, prolapsed cloaca; ST, swim bladder tear; HE, hemorrhage; OT, organ torsion; AE, arterial embolism.

[a]Internal signs of barotrauma were assessed in a subsample of line-caught rockfish recompressed to original capture depth in cages.

were no significant differences in the proportion of survivors among fish with or without each external sign of barotrauma (two-sided Fisher exact test, $p > 0.05$). However, the results of these analyses for stomach eversion were inconclusive because sample sizes within species for fish without stomach eversion were too small. When all species were pooled, there were significantly higher mortalities in fish having stomach eversion (33%) than in fish without (0%) (two-sided Fisher exact test, $p < 0.001$), with bocaccio and vermilion and squarespot rockfish comprising 62% of these mortalities. Externally, rockfish commonly showed the same five categories of external signs of barotrauma as reported in the initial capture survival experiment. Also similar to what was reported for initial capture survival, external barotrauma category was not a predictor of 2-day postrecompression survival, although the model results in this case were much more significant (logistic regression, $\chi^2 = 1.33744$, $df = 4$, $p = 0.855$).

A total of 51 rockfish were dissected to assess internal signs of barotrauma (survivors, $n = 36$; dead, $n = 15$) 2 days after being recompressed in cages. Fewer dead fish than live fish were dissected because 24% of the dead fish observed in cages were completely clean carcasses that could not be assessed. Overall, 50% of fish showing organ torsion suffered mortality compared with 28% of fish without organ torsion; however, this difference was not significant (two-sided Fisher exact test, $p = 0.22$). There were also no significant differences in percent mortality for fish with and without hemorrhage, swim bladder tears, or arterial embolism (two-sided Fisher exact test, $p > 0.05$). Sample sizes

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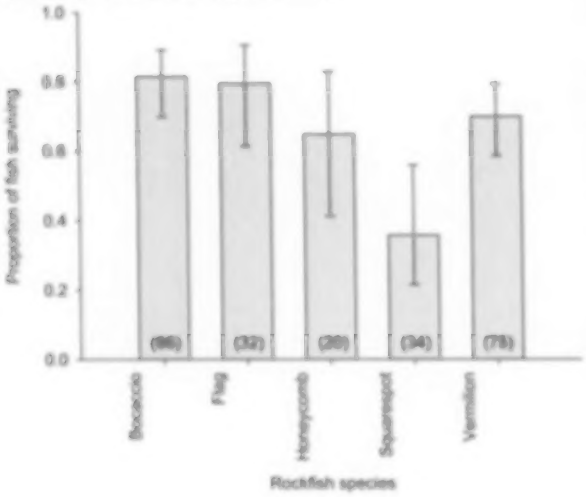
Fig. 3. Two-day survival (±95% binary confidence intervals) of the five most abundant species captured by hook-and-line in southern California from depths of 55–89 m and recompressed to original capture depth in cages (2-day postrecompression survival experiment, summer 2005 and 2006). Numbers in parentheses represent samples sizes. Percent survival was significantly different among species ($\chi^2 = 21.6$, df = 5, $p < 0.0001$).



were too small among groups of fish showing specific combinations of internal barotrauma to perform logistic regression analysis.

In the overall model, cage density, depth, and fish length had no significant effect on survival (Table 5) and, hence, were removed from the model. Mean values for each variable and species in the model are reported in Table 6. Species, surface holding duration, and temperature differential were significant predictors of survival in the reduced model (Table 5). Surface holding duration and temperature differential showed a negative relationship with the predicted probability of 2-day postrecompression survival (Fig. 4). The odds of mortality 2 days after recompression increased 1.7 times with every 10 min increase in surface holding time (e.g., $1/0.95^{10}$; Table 5). The odds of mortality 2 days after recompression increased 1.96 times with every 1 °C increase in seafloor–surface temperature differential (e.g., $1/0.51^{1}$).

There was no significant difference in median surface holding duration among species (Kruskal–Wallis, $H = 2.96$, $p = 0.56$) and no significant difference in average seafloor–surface temperature differential experienced among species (ANOVA, $F = 0.60$, $p = 0.66$).

Longer-term survival

Of the 125 fish released alive from cages at 20 m 2 days after capture, 3% were recaptured by local anglers near the areas where they were released. Two of eight honeycomb rockfish, two of 41 bocaccio, and one of 40 vermilion rockfish that were released alive were recaptured. Days at liberty for the recaptured honeycomb rockfish were 14 and 208 days. The third recaptured fish, a bocaccio, was reported after 28 days at liberty. The second recaptured bocaccio was reported approximately 90 days after the last tagging event; however, the angler provided no other additional information (i.e., tag number, date of capture) and so actual days at liberty are unknown. The vermilion rockfish was at liberty for 447 days.

Discussion

Barotrauma and initial capture survival

Despite species-specific differences in the types and degree of angling-induced barotrauma, most rockfish showed greater than 75% initial capture survival, suggesting that degree of barotrauma is not a good predictor of mortality within the first 10 min of capture. Other studies report high initial capture mortality as a result of gas bubble formation and exhaustion (Feathers and Knable 1983; Kieffer 2000; Stephens et al. 2002). However, in this study, the initial survival of rockfish overall did not appear dependent on the presence of gas bubbles. Beyer et al. (1976) reported that some bubbles present in the blood could be tolerated by coho salmon (*Onchorhynchus kisutch*), whereas large gas bubbles in vital areas such as the heart were lethal. Rosy and olive rockfish comprised the majority of dead fish with arterial embolism, suggesting these species may be more susceptible to lethal arterial embolisms than other species.

Exhaustion may restrict the ability of the opercular muscles to pump water past the gills and may also account for species-specific differences in initial capture mortality (Kieffer 2000). All of the olive rockfish and many rosy rockfish were moribund directly upon capture, suggesting that these two species may be susceptible to exhaustion. This exhaustion, in combination with the high incidence of arterial embolism observed in dead individuals, may account for the higher initial capture mortality of these two species relative to the other rockfish species.

Intraspecific variability in barotrauma responses of fish captured at similar depths are likely due to differences in the relative volume of the swim bladders when fish are caught (Arnold and Walker 1992; Rummer and Bennett 2005; Parker et al. 2006). For example, extent of barotrauma will vary depending on whether the fish is neutrally buoyant at the depth of capture (Parker et al. 2006). Further, interspecific variability in swim bladder morphology may also influence interspecific variation in the occurrence of swim bladder tears. During examination of internal signs of barotrauma, olive rockfish swim bladders were found to be relatively thin compared with the more robust swim bladders observed in vermilion, copper, and brown rockfish. Given the high occurrence of swim bladder tears in olive rockfish observed in this study relative to the other species, a thinner swim bladder may be more prone to severe rupture than a robust swim bladder, as seen in other species (Feathers and Knable 1983). Although olive rockfish showed high mortality and high occurrence of swim bladder tears, all other rockfish with swim bladder tears lived except for two ($n = 17$). Nevertheless, longer-term survival may be compromised by structural damage to the swim bladder and (or) other organs (Parker et al. 2006).

Barotrauma and 2-day postrecompression survival

The species-specific differences in external signs of barotrauma reported in this study appear to be related to species

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Table 5. Logistic regression results of the overall and reduced models of 2-day survival of rockfish captured by hook-and-line in southern California from depths of 55–89 m and recompressed in cages to original capture depth (2-day postrecompression survival experiment, summer 2005 and 2006).

Predictor of survival	df	β	X^2	p	OR
Overall model					
Species	4	—	11.23	0.024*	—
Surface time (min)	1	–0.626	9.69	0.002*	0.94
Depth (m)	1	–0.0277	2.22	0.136	0.97
Fish length (SL, cm)	1	0.0419	0.99	0.319	1.04
Cage density	1	–0.0462	0.26	0.614	0.96
Temperature difference (°C)	1	–0.6512	7.57	0.006*	0.52
Reduced model					
Species	4	—	19.76	0.001*	—
Surface time (min)	1	–0.0566	8.6312	0.003*	0.95
Temperature differential (°C)	1	–0.6705	8.4990	0.004*	0.51

Note: Asterisks (*) indicate significance at $p < 0.05$. df, degrees of freedom; OR, odds ratio; SL, standard length.

differences in body morphology and also to the degree of vertical movement within the water column. For example, species that showed few signs of barotrauma (e.g., bocaccio and squarespot, speckled, and halfbanded rockfish) can be characterized as having relatively elongate, laterally compressed bodies and occurring in schools up off the seafloor (Love et al. 2002). In contrast, flag, honeycomb, vermilion, and starry rockfish, which are all relatively deep-bodied and more demersal, showed a high degree of barotrauma. Species that show a high degree of barotrauma might be expected to have low survival following recompression relative to those species showing very few external signs of barotrauma; however, we did not observe this trend. Further, barotrauma category (e.g., specific combinations of external signs of barotrauma) was not a significant predictor of survival. Although the presence of specific external signs of barotrauma was not a clear indication of 2-day postrecompression survival, as has been found in other species (Gitschlag and Renaud 1994; Rummer and Bennett 2005), the absence of a specific sign of barotrauma may be more telling. For instance, 2-day postrecompression survival was significantly higher for fish without stomach eversion.

Stomach eversion may occur as a result of swim bladder leaks or ruptures. Although visible swim bladder tears were not common for the catch as a whole, we did observe many fish with partially deflated swim bladders, suggesting that escapement of gas at high pressure likely occurred during ascent. Although the mechanism by which swim bladders reseal after overinflation is not well understood, this may explain why not all rockfish with stomach eversion showed visible tears in the swim bladder wall. Delayed mortality in fish with stomach eversion may be a result of internal organ torsion associated with the occurrence of stomach eversion and (or) internal organ damage resulting from the overinflated swim bladder crushing organs (Keniry et al. 1996; Rummer and Bennett 2005). Fifty percent (4 of 8 fish) exhibiting organ torsion died versus only 26% (11 of 42 fish) without organ torsion. Low sample size among fish with organ torsion ($n = 8$) may explain why this difference was not significant. Further analyses of internal signs of barotrauma as predictors of postrecompression survival could not be conducted because of low sample sizes among dead specimens ($n = 15$).

Rummer and Bennett (2005) reported that internal organ damage of red snapper (*Lutjanus campechanus*) increased with depth of capture because of the progressive expansion of the swim bladder from posterior to anterior regions of the body cavity. Although not specifically tested, it is likely that swim bladder rupture thresholds and the space allowable for swim bladder expansion may also vary across rockfish species. Thus, the extent of organ damage in rockfish may differ among species with different body shapes and (or) swim bladder morphologies, even at similar capture depths. For example, squarespot rockfish (low survival) and bocaccio (high survival) frequently showed stomach eversion. Squarespot rockfish were observed as having thinner swim bladder morphology similar to that of olive, widow, and halfbanded rockfish, and three of six fish showed swim bladder tears. In contrast, bocaccio were observed to have relatively thicker swim bladders. Bocaccio swim bladders may rupture only minutely, allowing gas escapement into the body cavity at high pressure, which would force the stomach out of the mouth but nevertheless, leave the swim bladder visibly intact. Only one of 12 bocaccio showed swim bladder tears. It is important to note that swim bladder ruptures, although common in some species, may not be deleterious, at least in the short term. Several studies indicate a remarkable ability of physoclistic species to repair ruptured swim bladders within a few days or even hours (Burns and Restrepo 2002; Nichol and Chilton 2006; Parker et al. 2006).

In the initial capture survival experiment, the degree of barotrauma in rockfish was partially attributed to depth; however, depth was not a significant predictor of 2-day postrecompression survival of rockfish. Depth has been shown in numerous studies to significantly affect postrelease survival in other physoclistic species (Wilson and Burns 1996; Morrissey et al. 2005; St. John and Syers 2005). Nevertheless, because rockfish exhibit a wide range of foraging behavior (e.g., benthic ambush predators versus water-column planktivores) and were observed to differ in swim bladder morphology as discussed above, depth effects on rockfish survival corresponding to the incidence and degree of barotrauma are likely to differ by species (Lea et al. 1999; Parker et al. 2006; Hannah and Matteson 2007).

In addition to depth, fish size was not a significant predictor of 2-day postrecompression survival. It has been hypothesized that smaller fish may be more susceptible to gas embolism because of the relationship between critical blood vessel size and the size of expanded bubbles in the blood (Beyer et al. 1976), and thermal shock may occur more quickly in smaller fish than in larger fish (Davis 2002); however, other studies report no effect of fish length on short-term survival (Gitschlag and Renaud 1994; Collins et al. 1999).

Cage density was not found to affect 2-day postrecompression survival; nevertheless, we cannot dismiss that

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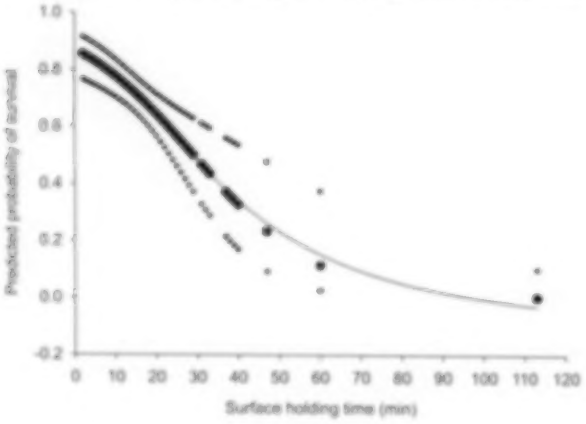
Table 6. Species catch-and-release summary data (mean ± standard deviation, SD) for the five most abundant rockfish captured by hook-and-line in southern California and recompressed to original capture depth in cages (2-day postrecompression survival experiment, summer 2005 and 2006).

Common name	Standard length (cm)	Depth (m)	Cage density	Temperature differential (°C)	Surface holding duration (min)
Bocaccio	35.2 (7.4)	83.3 (4.3)	7 (2)	9.7 (0.7)	14.4 (6.7)
Flag rockfish	21.5 (2.7)	69.9 (13.1)	8 (2)	9.4 (0.6)	13.6 (9.3)
Honeycomb rockfish	16.3 (1.7)	76.7 (10.8)	8 (3)	8.2 (0.7)	14.9 (9.1)
Squarespot rockfish	19.9 (1.3)	70.2 (12.0)	7 (2)	9.3 (0.7)	21.5 (21.2)
Vermilion rockfish	24.3 (4.0)	63.8 (11.5)	7 (2)	9.0 (1.0)	16.0 (9.7)
Overall mean (±1 SD)	26.0 (8.1)	72.4 (12.9)	7 (2)	9.2 (0.9)	15.8 (11.3)

Fig. 4. Predicted probability (±95% confidence interval) of rockfish 2-day postrecompression survival as a function of surface holding time (min) (2-day postrecompression survival experiment, summer 2005 and 2006). The probability curve is based on the five most abundant rockfish captured during the 2-day cage trials; $n = 211$ fish).



observed cage abrasion and potentially high stress levels due to the confinement of the cages may have contributed to some mortality (Gliniak et al. 2006). The presence of scavengers such as crustacean zooplankton or hagfish, as suggested by the clean carcasses of several fish, may have further exacerbated stress levels in other fish that had not suffered immediate mortality (Stepien and Brusca 1985).

Seventy-five percent of fish showed cloudy corneas 2 days after recompression, but fish were observed bumping into the sides of the cages during cage retrievals. We conclude that cloudy corneas were likely a symptom of keratitis as a result of cage abrasion because even fish without initial ocular trauma exhibited cloudy corneas after recompression (St. John and Syers 2005). Recompression appeared to reverse or alleviate external signs of barotrauma observed in rockfish upon capture, which has also been noted in previous studies (St. John and Syers 2005; Parker et al. 2006; Hannah and Matteson 2007). Still, it is unclear whether ocular trauma may result in long-term visual impairment.

Rapid recompression of line-caught rockfish can reduce the extent of injury caused by arterial embolism and hemorrhaging and thereby increase survival. In this study, surface holding time was found to have a significant effect on recompression survival. Fish held at the surface for 10 min or less had a 78% probability of survival following recompression, and this probability of survival increased to 83% if fish were released within 2 min of landing. Parker et al. (2006) reported 97% survival (up to 21 days) of black rockfish (*Sebastes melanops*) recompressed within 30 s of decompression (from 4 atm) in pressure chambers. Surface holding duration may explain species-specific differences in survival in our study. Of the five most abundant species caught (e.g., bocaccio and vermilion, flag, honeycomb, and squarespot rockfish), squarespot rockfish showed the lowest survival. It is possible that surface holding duration may have affected squarespot rockfish survival because these fish were held at the surface an average 5 min longer than the other four species (Table 6). This difference in surface holding duration, although not statistically significant, may have been biologically significant.

Fish held at the surface for long periods of time may also experience thermal stress (Feathers and Knable 1983). Reduced blood flow as a result of intravascular bubble formation following decompression may be especially detrimental in warmer surface waters where oxygen demand is higher and oxygen concentration is lower. Feathers and Knable (1983) reported a synergistic effect of barotrauma and surface temperature on mortality of released largemouth bass (*Micropterus salmoides*). Because of the physiological effects of temperature on rockfish and the negative affect of increased temperature differential on short-term survival, it is possible that postrelease survival probabilities relative to surface holding duration may increase during winter months when sea surface temperatures differ less from seafloor temperatures.

Longer-term survival

Although tag recaptures were few, they provided evidence of longer-term postrelease survival (at least up to 1.5 years) in line-caught rockfish following recompression. Although these data provide no indication of actual delayed mortality following release, other studies document delayed capture mortality following recompression up to the first several days of release (Gitschlag and Renaud 1994; Wilson and Burns 1996; Lowe et al. 2007). For example, 93% of line-caught rockfish that were acoustically tagged at the surface, recompressed to depth, and monitored for a 2-year period were detected within the first 2 days after their release (Lowe et al. 2007). Subsequently, the decline in detections leveled out 6 days after release. Cage recompression studies on line-caught red snapper and red grouper (*Epinephelus morio*) found that delayed mortality continued up to 10 days, although the majority of deaths occurred within

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the first 2 days (Gitschlag and Renaud 1994; Wilson and Burns 1996).

Fisheries management implications

The results of this research provide evidence of both short-term and long-term postrelease survival of line-caught southern California nearshore and shelf rockfish recompressed to capture depth (from 55 to 89 m). Findings suggest that the utility of recompression devices is high if used within minutes of capture. A variety of assisted release (i.e., recompression) methods, including inverted weighted milk crates and "fish descenders", currently exist to return overinflated fish to depth following capture (Theberge and Parker 2005). Although the use of recompression devices would not be practical for a strictly catch-and-release fishery, recompression would provide a practical means for decreasing discard mortality of incidentally caught rockfish.

Between 1993 and 2002, an estimated two million rockfish were discarded in southern California (Pacific States Marine Fisheries Commission 2007). Fifty-eight percent of fish were discarded from private boats. Dwarf species (i.e., honeycomb, squarespot, and halfbanded rockfish) comprise a large proportion of rockfish discards (M. Horeczko, California Department of Fish and Game, 4665 Lampson Avenue, Los Alamitos, CA 90720, USA, personal communication), which likely suffer high mortality. These species are now among the common rockfish caught in southern California (Love et al. 1998), although they are seldom kept by fishers because of their small size. The practice of using assisted release in the recreational rockfish fishery may help restore rockfish diversity on rocky reefs that have suffered decades of intense fishing pressure, which in turn would help to increase ecosystem function and resilience to fishery collapse (Worm et al. 2006).

Minimum size limits have not been considered as a management tool for rockfish because of the perceived high release mortality attributed to barotrauma (J. Ugoretz, California Department of Fish and Game, 20 Lower Ragsdale Drive, Monterey, CA 93940, USA, personal communication), but federal and state mandates outlined in the Magnuson Fishery Conservation and Management Act (MFCMA) [301 (a)(9)] and the Marine Life Management Act (MLMA) [7056(d)] require a reduction in groundfish bycatch (i.e., incidental catch + discards) mortality. Some studies have evaluated the effectiveness of deflating swim bladders to increase survival of overinflated fish by relieving excess gas with a hypodermic needle ("venting"), which enables fish to swim to depth on their own following release (Bruesewitz et al. 1993; Keniry et al. 1996; Collins et al. 1999). However, venting is still controversial because the results of these studies are not definitive and experience in using this method is necessary to avoid accidental puncture of vital organs (Kerr 2001). Implementation of less invasive assisted release methods would increase the practicality of size limits as an alternative to current nearshore and shelf rockfish regulations. Nevertheless, for minimum size limits to be effective in conserving rockfish stocks, fish that are recompressed to depth after capture must live long enough to reach size at maturity. For long-lived species such as rockfish, it may be important to consider cumulative mortality risk, which increases exponentially with every recapture (Bartholomew and Bohnsack 2005). Based on rockfish recapture rates from this study and those reported from the Nearshore Groundfish Tagging Project, rockfish recapture rates in southern California appear low (3%; Hanan and Associates Inc. 2006). Furthermore, the possibility of subsequent mortality of a recaptured immature fish is likely offset by the decrease in overall fishing mortality that would be provided by implementing recompression. Other factors, including vision impairment and (or) angling-induced physiological impacts on growth and reproduction that may decrease rockfish longevity following capture and recompression, need further exploration.

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Diet overlap and prey selection through metamorphosis in Irish Sea cod (*Gadus morhua*), haddock (*Melanogrammus aeglefinus*), and whiting (*Merlangius merlangus*)

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Abstract: Copepods in the genus *Calanus* are usually considered to be the preferred prey of gadoid larvae in many areas; however, in the Irish Sea, the abundances of these *Calanus* species are low and highly variable. We use this situation to test whether *Calanus* species are still actively selected by gadoid larvae in *Calanus*-poor environments. Diets of Irish Sea cod (*Gadus morhua*), haddock (*Melanogrammus aeglefinus*), and whiting (*Merlangius merlangus*) were studied from the yolk-sac stage to the juvenile stage. Prey from stomach contents were compared with in situ abundance via an index of prey preference. As expected, all larvae selected copepod nauplii at the onset of feeding. As the larvae developed, their prey preferences changed and varied with species. Cod and whiting showed a similar transition of prey species preference, with a clear preference for *Calanus* species after metamorphosis, even in this area of low abundance of these *Calanus* species. The diet composition of haddock differed from that of cod and whiting, as nauplii remained in their diet later into development and there was little preference for individual copepod species detected. The differences in prey selectivity suggested between these gadoids may be attributed to their population variability through the known variability of their preferred prey items.

Résumé : On considère généralement que les copépodes du genre *Calanus* constituent les proies de prédilection des larves de gadoïdes dans plusieurs régions; dans la mer d'Irlande, cependant, les densités de ces espèces de *Calanus* sont faibles et très variables. Cette situation nous a permis de vérifier si les espèces de *Calanus* sont recherchées de façon active par les larves de gadoïdes dans les milieux pauvres en *Calanus*. Nous avons étudié le régime alimentaire de morues (*Gadus morhua*), d'aiglefins (*Melanogrammus aeglefinus*) et de merlans (*Merlangius merlangus*) depuis l'alevin vésiculé jusqu'au stade juvénile dans la mer d'Irlande. La comparaison des proies dans les contenus stomacaux avec les densités in situ s'est faite à l'aide d'un coefficient de préférence des proies. Comme prévu, toutes les larves sélectionnent les nauplies de copépodes au début de leur période d'alimentation. À mesure que les larves se développent, leur préférence de proies change et varie d'une espèce à l'autre. Les morues et les merlans connaissent le même changement de préférence des proies, avec une nette prédilection pour les espèces de *Calanus* après leur métamorphose, même dans cette région de faibles densités de ces espèces de *Calanus*. La composition du régime alimentaire des aiglefins diffère de celle des morues et des merlans, puisque les nauplies demeurent dans le régime plus tard durant le développement et qu'il ne se manifeste que peu de préférence pour des espèces particulières de copépodes. Ces différences dans la sélectivité des proies qui semblent exister entre ces gadoïdes peut s'expliquer par la variabilité de la population consécutive à la variabilité connue chez leurs proies préférées.

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Introduction

Recruitment ultimately relies on larval survival, and therefore it is important to understand the dynamic relationship between fish larvae, their environment, and the processes that drive their survival and growth (Houde 1987, 1989). The larval prey field (availability and quality) has been suggested as an important factor in the determination of survivorship of larvae from Hjort's (1914) "critical period" to Cushing's (1975) "match mismatch" hypothesis. Larvae become more adept at locating, consuming, and digesting prey items as they grow and develop (Fuiman and Higgs 1997). Thus, larval growth and development, as well as species preferences, are all aspects of the dynamic interactions between fish larvae and zooplankton prey. For example, among gadoids in the North Atlantic, the diets during early larval stages consist mainly of the nauplii of copepods (Last 1978; Kane 1984; Economou 1991). With increasing larval size and increasing gape size, larger prey items are taken, and often the large calanoid *Calanus* spp. dominates the diets of larger larvae (Last 1978; Thorisson 1989; Economou 1991). The diet overlap between gadoid species has been estimated at >75% (Kane 1984).

The selection of prey taken by larvae is often described by comparison of differences in the proportions of the prey types or sizes that are consumed and those that are available in the environment (Eggers 1977; Juanes and Conover 1994), and a number of different methods are available for calculating prey selectivity (Schoener 1971; Chesson 1978). However, predator selection of prey must take into account prey availability, digestibility, and perception (Wootton 1990), as well as other factors. Larvae become better predators (Hunter 1980; Fuiman and Higgs 1997), and selectivity may change as larvae grow. Very little work has been done on prey selection in relation to larval development, especially at metamorphosis, an important life phase as individuals undergo both physiological and morphological changes during this period (Kjørsvik et al. 1991; Osse and van den Boogaart 1995; Fuiman and Higgs 1997). These changes require a higher energetic input, as suggested by raised gut clearance rates, therefore the selection of diet is particularly important as there may be an increased risk of starvation (Jordaan and Brown 2003). With the suggestion that cod are less able to feed during metamorphosis (Thorisson 1994), higher mortality rates could therefore occur at lower prey abundance.

The aim of this study is to improve our understanding of the interactions between gadoid larvae and their prey environment. Cod (*Gadus morhua*), haddock (*Melanogrammus aeglefinus*), and whiting (*Merlangius merlangus*) are important, commercially exploited whitefish in the Irish Sea (Armstrong and Dickey-Collas 1997; International Council for the Exploration of the Sea (ICES) 2006). In many geographic regions, *Calanus* spp. are the dominant prey item in the diet of larvae gadoids (Heath and Lough 2007), but the Irish Sea is an area of low *Calanus* spp. abundance (Gowen et al. 1997; Nash and Geffen 2004), being dominated by smaller copepod species. This region is thus an ideal model in which to compare the feeding and prey selectivity of young gadoids versus those in the North Sea, Northeast Atlantic, or Barents Sea where *Calanus* spp. is in much higher abundance (Conover 1988; Heath et al. 2000; Beaugrand et al. 2003). The low abundance of *Calanus* spp. in the Irish Sea may lead to different selectivity patterns as the larvae utilize the smaller, dominant copepod species as the principal source of food. Pepin and Penney (1997) suggested that larvae with a more flexible foraging behaviour would be less vulnerable to changes in prey abundance or availability of particular size categories of prey, and this may be more readily identified by comparing the diets of larval gadoid species over a range of prey situations. The aim of this study is therefore to compare and contrast prey selection between cod, haddock, and whiting in an environment of low *Calanus* abundance and to measure changes in prey selection in relation to larval development.

Materials and methods

Sample collection and preservation

Samples of gadoid larvae and zooplankton were collected at three stations in April 2001 and at four stations in May 2001 in the northwestern Irish Sea from the Fisheries and Aquatic Ecosystems Branch, Agri-Food and Biosciences Institute – Northern Ireland (AFBI) research vessel M.R.V. *Lough Foyle*. The full range of gadoid developmental stages, yolk-sac larvae to juveniles, were obtained by sampling with a multiplankton (MP) sampler with 280 μm mesh (Hydrobios Apparatebau GmbH, Kiel, Germany; Weikert and John 1981), a MOCNESS sampler (multiple opening and closing net and environmental sensing system; Wiebe et al. 1976; Roe and Shale 1979) with 1000 μm mesh, and a German pelagic juvenile trawl (PT). The first two samplers were towed for 20 min using a stepwise oblique profile at 3 kn (1.5 m·s^{-1}). The pelagic trawl was towed for 20 min at between 4 and 5 kn (2–2.6 m·s^{-1}). Comparable zooplankton samples were obtained using the MP sampler and a GULF VII high-speed plankton sampler (Nash et al. 1998) of 280 μm mesh size with a PUP net attachment of 60 μm mesh size. The GULF VII was towed using a double oblique profile for 20 min at a speed of 3 kn (1.5 m·s^{-1}). Sampling at each station took place over a 24-h period, where numerous tows of the different sampling equipment were carried out (see summary in Table 1).

The sampling strategy throughout the study was designed to minimize the problems of patchy distribution of zooplankton and fish larvae (Omori and Hamner 1982). The MP sampler captured both gadoid larvae and their zooplankton prey simultaneously. The mesh size of the MOCNESS and PT was too large to catch most zooplankton, so for the gadoids caught with these samplers, comparative zooplankton abundance and composition were determined from the MP, GULF VII, and PUP samples taken closest to the time of the MOCNESS and PT deployment. This meant that comparative gadoid and zooplankton samples were never taken with more than a 3-h separation between sampling.

All samples were preserved immediately in 4% buffered (sodium acetate trihydrate) formaldehyde. For the gadoid larvae, this was to reduce larval shrinkage (Blaxter 1971; Hay 1981) and rapidly harden body tissue, which stopped digestion of prey items in the guts for easier identification (Bowen 1996).

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Table 1. Summary of gadoid and zooplankton sample collection methodology during April and May of 2001 in the northwestern Irish Sea.

			Samples taken between 23 and 26 April		Samples taken between 14 and 17 May	
Station	Station depth (m)	Gear type	No. of tows	Sampling depths (m)	No. of tows	Sampling depths (m)
1	44	MP	16	5 + 20 + 35	6	5 + 25 + 40
		GULF+PUP	3	All	1	All
		ROSETTE	2	5 + 20 + 35	2	10 + 25 + 35
		MOCNESS	*		2	35
		PT	*		1	All
2	95	MP	16	10 + 30 + 50 + 80	12	10 + 30 + 50 + 80
		GULF+PUP	3	All	3	All
		ROSETTE	2	10 + 30 + 50 + 80	2	15 + 30 + 50 + 80
		MOCNESS	*		2	10 + 30 + 50
		PT	*		1	All
3	83	MP	17	10 + 30 + 50 + 70	12	10 + 30 + 50 + 70
		GULF	3	All	3	All
		ROSETTE	2	10 + 30 + 50 + 70	2	10 + 30 + 50 + 70
		MOCNESS	*		7	10 + 30 + 50 + 70
		PT	*		1	All
4	47	MP	*		10	5 + 25 + 40
		GULF	*		3	All
		ROSETTE	*		2	5 + 25 + 40
		MOCNESS	*		1	40
		PT	*		1	All

Note: Sampling equipment: Hydrobios multiplankton (MP) sampler (Weikert and John 1981); multiple opening and closing net and environmental sensing system (MOCNESS) sampler (Wiebe et al. 1976; Roe and Shale 1979); German pelagic juvenile trawl (PT) and GULF VII high-speed plankton sampler (Nash et al. 1998) with PUP net attachment (GULF+PUP); water bottles attached to a rosette sampler (ROSETTE). An asterisk (*) denotes that a sample was not taken.

Anatomical measurements

The standard lengths of all gadoids were measured either under a dissecting microscope with graticule or with vernier calipers (with a precision of 0.05 mm) in the case of juveniles. However, no juveniles larger than 40 mm in length were included in this study as benthic feeding commences at this size (Hüssy et al. 1997; Lomond et al. 1998) and no comparable benthic prey samples were taken.

The gadoids were assigned to one of three developmental stages based on standard lengths as described by Rowlands et al. (2006). These stages were larval (<8 mm), intermediate (8–14 mm), and juvenile (>14 mm).

Gut content analysis

Once standard lengths had been measured, the stomach of each larva was removed. In gadoid larvae, the stomach does not become differentiated until a standard length of ~15 mm (Economou 1991). Therefore, in larvae in which the stomach was not obvious, the anterior part of the alimentary canal (the oesophagus to the first point of inflection of the intestine) was removed for analysis; however, when the stomach was easily identified, only this and the oesophagus were removed.

The gut was opened using forceps and a scalpel for larger stomachs or insect needles attached to 2 mm diameter glass tubes for smaller specimens. The contents of each gut were extracted by peeling back the gut wall and washing out the prey items into a Petri dish using distilled water. Prey items contained within the gut were identified to the lowest taxonomic level possible. However, because of the difficulties in identifying some copepod species after ingestion and to allow later comparisons, *Calanus finmarchicus* and *Calanus helgolandicus* were grouped and referred to as *Calanus* spp., and *Pseudocalanus elongatus* and *Paracalanus parvus* were also grouped and referred to as *Pseudo/Paracalanus*. This same grouping of species was used in the in situ zooplankton identifications.

Zooplankton analysis

To study selection in gadoid feeding, the proportions of each prey group in the gut were compared with the proportions of these prey items in the environment (Chesson 1978). The MP and GULF VII samplers were used to estimate the abundances of the larger-sized zooplankton. However, a 200 µm mesh used in the capture of fish larvae may capture as little as 10% of the mesoplankton (Graziano 1988; Gallienne and Robins 2001) as smaller organisms passed through the mesh. A net that adequately collects adult copepods would be too coarse to sample nauplii, and if a smaller mesh is used to collect all stages, it will catch nauplii and copepods at different efficiencies (Mauchline 1998). Therefore, in this study, a PUP net (60 µm mesh) attachment to the GULF VII sampler was used to estimate the abundances of smaller organisms present in the water column (particularly nauplii and *Oithona* spp.). To calculate the total proportions of all zooplankton in the environment, the PUP, GULF VII, and MP data had to be combined. As samplers were deployed at the same times, this was done by assuming that the sampler with the largest abundance of a particular organism gave the true abundance.

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Data and statistical analysis

To study diet overlap between the gadoid species, two methods were used. To compare all larvae individually, Schoener's (1970) overlap index was used:

$$\alpha = 100\left(1 - (0.5\sum_{i=1}^{n} |P_{xi} - P_{yi}|)\right)$$

where α is the index of overlap, P_{xi} and P_{yi} are the proportions of food category i in the diets of species x and y, respectively, and n is the total number of food categories observed. Secondly, diet overlap was investigated using multivariate techniques. Gut contents of individual fish were compared by studying the percentage of prey species composition. These data were looked at using both principal components analysis (PCA) and canonical variate analysis to find and display patterns of resemblance in the gut content data between the three species of gadoids.

The prey selectivity of the different stages and species of gadoid larvae was expressed through the maximum likelihood estimator and an electivity index. First, preference was calculated using the maximum likelihood estimator (ML) ($\hat{\alpha}_i$) (Chesson 1978), which assumes that there is no depletion of food resources as a result of feeding (a reasonable assumption as grazing by larvae on zooplankton probably has little effect on the zooplankton population (Cushing 1983; Pepin and Penney 2000)):

$$\hat{\alpha}_i = \frac{r_i/n_i}{\sum_{i=1}^{m} r_i/n_i}, \quad i = 1, \ldots, m$$

(Chesson 1978), where r_i is the proportion of food item i in the consumer's stomach, n_i is the proportion of food item i in m, where m is the total number of food items. The advantage of this method of quantifying preference is that ML estimators are asymptotically distributed as normal variables and the preferences are likely to vary about a population mean. Therefore, the value of $\hat{\alpha}$ can be regarded as a sample from a multivariate normal distribution and so standard statistical tests can be carried out on the data (Chesson 1983). However, if a predator was selecting food at random ($\alpha_i = 1/m$), the value of α_i would vary with the number of food types available (m), so it is not easy to visualize the strength of preference for a particular item between different environmental prey concentrations. Therefore, a second method of measuring prey selectivity using an electivity index (ε_i), which ranges from −1 to +1, with 0 representing no preference (Chesson 1983), was employed for display purposes. However, because of the properties of ε_i, it cannot be used for parametric analysis:

$$\varepsilon_i = \frac{m\alpha_i - 1}{(m-2)\alpha_i + 1}$$

The majority of comparisons were carried out using analysis of variance (ANOVA) after determining normality and homogeneity of variance. The preference data, which were formulated as proportions, were arcsine-transformed to allow parametric comparisons of the data. Regression analysis (generalized linear model, GLM) was used to compare predator–prey length data, again after determining homogeneity of variance and normality.

Results

Diet overlap between species

A total of 130 cod, 184 haddock, and 351 whiting were caught, and considerable overlap in diets was seen between both developmental stages and species. Using multivariate analysis, overlaps were clearly seen by studying canonical analysis score plots with centriod values and 95% confidence intervals (CIs) (Fig. 1). Interspecific overlap declined throughout development (Figs. 1*a*–1*c*), shown by a reduction in overlaps of the 95% CIs between gadoid species. The diet overlap between haddock and either cod or whiting declined most rapidly with development. This overlap was also lower than that observed between cod and whiting, with the exception of the "larval" stage, when overlap between all species was very high. At each developmental stage, haddock consumed a greater array of prey species, as demonstrated by larger 95% CI bands for prey compared with those of cod and whiting. This suggested a wider prey species niche size in haddock vs. cod and whiting.

In the larval stage (Fig. 1*a*), the strongest factor (factor 1) suggested that an increase in nauplii numbers in the gut was correlated with a decrease in numbers of all other prey items, as noted by the large positive value for nauplii in comparison with the negative values for the other prey items (Table 2). In the intermediate stage (Fig. 1*b*), an increase in the numbers of midsized copepods (especially *Pseudo/Paracalanus*) correlated with a decrease in the proportion of smaller organisms (notably nauplii) (Table 2), whereas in the juvenile stage (Fig. 1*c*), it appeared that an increase in the number of large prey items was correlated with a decrease in the number of midsized copepods (Table 2). However, less than 40% of the diet overlap was explained by the first two factors of the multivariate analysis (Table 2).

Intraspecific diet overlap during the different development stages was also examined (Figs. 1*d*–1*f*). In both cod and haddock, the greatest overlap was between the intermediate and larval (I/L) stages, with less overlap between the intermediate and juvenile (I/J) stages, and no overlap observed between larval and juveniles (L/J) stages. However, in whiting, diet overlap was observed among all stages, although it was higher between the I/L and I/J stages. In all instances (Figs. 1*d*–1*f*), factor 1 suggested a negative correlation between nauplii and all other prey items, whereas factor 2 showed little pattern (Table 2), though less than 50% of diet overlap was explained by these two factors.

Schoener's (1970) index of overlap showed similar patterns. For each species studied (cod, haddock, and whiting), intraspecific overlap decreased significantly through development (cod, $F_{[2,558]} = 416.1$, $p << 0.001$; haddock, $F_{[2,978]} = 1515.1$, $p << 0.001$; whiting, $F_{[2,370]} = 1196.3$, $p << 0.001$) (Fig. 2). These results were comparable with those from the multivariate analysis, with the exception that in all three species, the values of Schoener's index indicated that there was also diet overlap between the larval and juvenile stages of all gadoid species.

When developmental stages were compared between spe-

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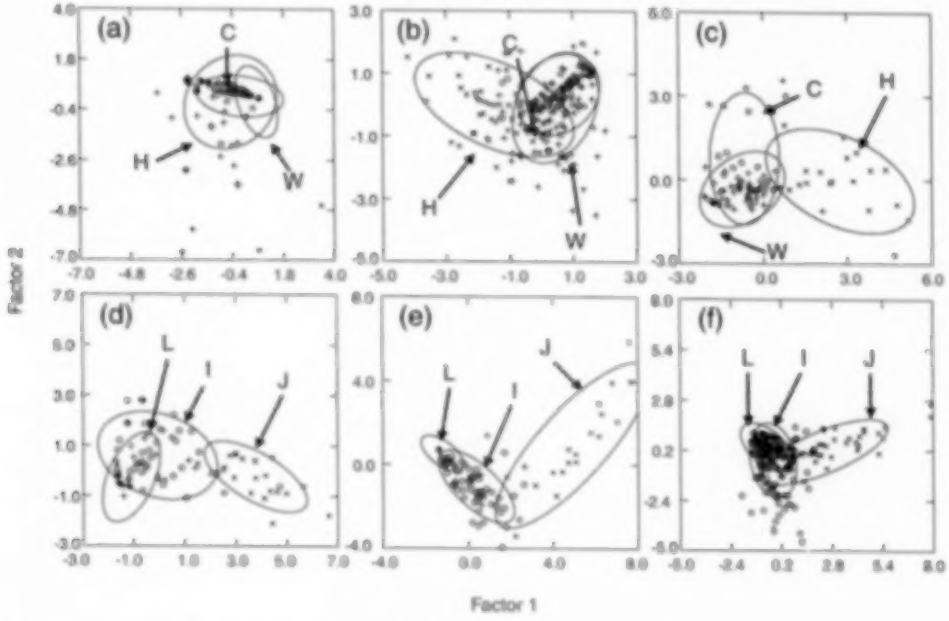
Fig. 1. Canonical variate analysis score plots for the first two factors explaining the majority of stomach content data variation. Ninety-five percent confidence intervals (rings) around a centriod (not displayed) are shown to indicate the diet ranges shown between larvae (L), intermediate (I), and juvenile (J) stages and cod (C), haddock (H), and whiting (W) gadoid species. In (*a*–*c*), haddock are represented by ×, cod by open circles, and whiting by +; in (*d*–*f*), juveniles are represented by ×, intermediates by open circles, and larvae by +.



cies, Schoener's index indicated that diet overlap at the L/I stage was greatest in haddock ($F_{[2,31373]}$ = 106.77, $p \ll 0.001$) at 47.2% ± 0.8%. However, at the I/J and L/J stages, the diet overlap was lowest in haddock at 16.5% ± 1.0 and 6.5% ± 0.8%, respectively ($F_{[2,9911]}$ = 375.0, $p \ll 0.001$; $F_{[2,11047]}$ = 185.9, $p \ll 0.001$, respectively). No significant difference in diet overlap was seen between cod and whiting at any developmental stage. Interspecific diet overlap also changed with development (Fig. 3). In the larvae, the lowest diet overlap was observed between cod and whiting at 51.6% ± 0.85 ($F_{[2,28305]}$ = 448.7, $p \ll 0.001$) compared with their overlaps with haddock (69.6% ± 0.9% and 58.1% ± 0.7%, respectively). However, in both the intermediate and juvenile stages, comparisons between cod and whiting showed the greatest overlap ($F_{[2,21266]}$ = 226.6, $p \ll 0.001$; $F_{[2,2467]}$ = 504.8, $p \ll 0.001$). Interspecific diet overlap was seen to decrease with development, with the exception of cod and whiting, which had the greatest overlap (54.9% ± 1.6%) in the juvenile stage.

Prey preference

During the larval stage, cod, haddock, and whiting selected for nauplii (Fig. 4*a*) in preference to all other prey items ($F_{[6,608]}$ = 143.3, $p \ll 0.001$; $F_{[6,384]}$ = 94.3, $p \ll 0.001$; $F_{[6,811]}$ = 75.8, $p \ll 0.001$, respectively). The preference for nauplii decreased with increasing larval length and developmental stage (Fig. 4*a*). Gadoids in the intermediate stage of development increasingly preferred copepods, although few copepod species were positively selected for until the start of the juvenile stage (Figs. 4*b*–4*f*). In cod, haddock, and whiting, preference for *Pseudo/Paracalanus* started to increase in the larval stage and continued into the intermediate stage. In the intermediate stage in both cod and whiting, *Acartia clausii*, *Calanus* spp., and *Oithona similis* were also increasingly preferred. In haddock only, *O. similis* increased in preference and to a higher level than in both cod and whiting ($F_{[2,279]}$ = 5.94, $p < 0.01$). However, in all cases, although preference for these species increased, the index of electivity was still negative, so prey species were less prevalent in the diet than in the plankton. These observations along with the fact that haddock had the greatest preference for nauplii while in the intermediate stage suggests that the diet preferences of haddock diverge from the similar diets of cod and whiting as they develop.

In the juvenile stage, there was positive preference for adult copepods. Initially cod (14–22 mm standard length) strongly preferred *O. similis* and *A. clausii*. The strength of this preference was significantly greater for cod than for the other two gadoid species ($F_{[2,92]}$ = 5.43, $p < 0.01$; $F_{[2,92]}$ = 8.37, $p \ll 0.001$, respectively). Cod juveniles larger than 22 mm also preferred *Pseudo/Paracalanus*, followed by *Calanus* spp., until a length of 40 mm, at which length *Calanus* spp. were the only species positively selected for. This pattern seen in cod was similar to that observed for whiting, with the exceptions that *O. similis* was strongly selected against, *A. clausii* was not as strongly preferred, and positive preference of *Pseudo/Paracalanus* occurred at a length of 16 mm. Finally, in haddock, there was no positive preference for any adult copepods until the fish reached a length of 36 mm, when both *Calanus* spp. and *T. longicornis* were weakly selected for. Each of the gadoids studied showed positive preference for *Calanus* spp. towards the end of the

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Table 2. Results of principal component analysis (PCA) of standardized gut content data showing Eigenvalues (EV), percentage of variation explained by each factor (% x2), and PCA variable loadings for interspecific comparisons (Comp.) for (*i*) larvae and (*ii*) intermediate and (*iii*) juvenile stages along with intraspecific developmental comparisons for (*iv*) cod, (*v*) haddock, and (*vi*) whiting.

Comp.	Factor	EV	% x2	*Acartia clausi*	*Calanus* spp.	*Metridia lucens*	*Paracalanus*/*Pseudocalanus*	*Temora longicornis*	Other calanoid copepods	*Oithona similis*	Other harpacticoid copepods	Copepod naplii
i	1	2.16	21.61	−0.01	−0.04	0.00	**−0.47**	**−0.23**	**−0.53**	−0.09	0.00	**0.64**
	2	1.14	11.45	**−0.36**	**−0.12**	0.00	0.07	**0.40**	0.05	**0.45**	0.00	**0.12**
ii	1	1.56	14.30	0.06	**0.15**	0.00	**0.53**	**0.10**	**0.58**	**−0.17**	**−0.10**	**−0.36**
	2	1.88	12.57	**0.20**	**−0.04**	0.00	**−0.27**	**−0.15**	**0.24**	−0.04	0.00	**−0.66**
iii	1	3.24	24.95	0.00	**0.12**	**0.30**	**−0.39**	**0.34**	**−0.36**	0.01	0.00	0.01
	2	2.51	19.33	**0.47**	**−0.26**	−0.09	**−0.35**	**−0.19**	−0.09	**0.44**	0.00	**0.45**
iv	1	2.60	25.95	**0.32**	**0.41**	0.00	**0.42**	**0.19**	**0.23**	**0.29**	0.00	**−0.52**
	2	1.62	16.17	**−0.49**	0.04	0.00	**0.32**	**0.30**	**0.16**	**−0.38**	0.00	**−0.22**
v	1	3.68	26.31	0.02	**0.39**	**0.25**	**0.23**	**0.40**	0.06	0.01	0.00	**−0.80**
	2	1.70	12.10	**0.16**	−0.07	**−0.33**	**0.35**	0.00	**0.46**	**0.36**	0.00	**−0.36**
vi	1	2.56	19.65	−0.02	**0.44**	0.00	**0.39**	**0.34**	**0.14**	−0.08	−0.04	**−0.41**
	2	1.48	11.36	**0.58**	**−0.39**	0.00	0.06	**−0.11**	**0.41**	**0.40**	−0.07	**−0.34**

Note: Bold values indicate significant results. Cod, *Gadus morhua*; haddock, *Melanogrammus aeglefinus*; whiting, *Merlangius merlangus*.

Fig. 2. Index of overlap (Schoener 1970) with associated 95% confidence intervals between larval and intermediate (L/I), intermediate and juvenile (I/J), and larval and juvenile (L/J) developmental stages for cod (*Gadus morhua*; solid bars), haddock (*Melanogrammus aeglefinus*; hatched bars), and whiting (*Merlangius merlangus*; open bars).

Fig. 3. Index of overlap (Schoener 1970) with associated 95% confidence intervals between cod and haddock (C/H), cod and whiting (C/W), and haddock and whiting (H/W) for larval (solid bars), intermediate (hatched bars), and juvenile (open bars) developmental stages. Cod, *Gadus morhua*; haddock, *Melanogrammus aeglefinus*; whiting, *Merlangius merlangus*.

juvenile stage at 40 mm (as defined in this study). Even though selection for *Calanus* spp. appeared to be much greater by both cod and whiting compared with haddock, when the stage was analysed as a whole, no significant differences were observed.

Preference for the large copepod *Metridia lucens* was strongly negative at every larval developmental stage and by each species of gadoid. This copepod is comparable in size with *Calanus* spp. and could otherwise be assumed to be attractive to juvenile gadoids when they are preying on larger copepods.

Discussion

The stomach contents of Irish Sea gadoid larvae were similar to those of previous larval diet studies from other areas (Economou 1991; Pepin and Penney 1997; Heath and Lough 2007) in which nauplii and calanoid copepods made up the bulk of the diet. However, whereas Marak (1960)

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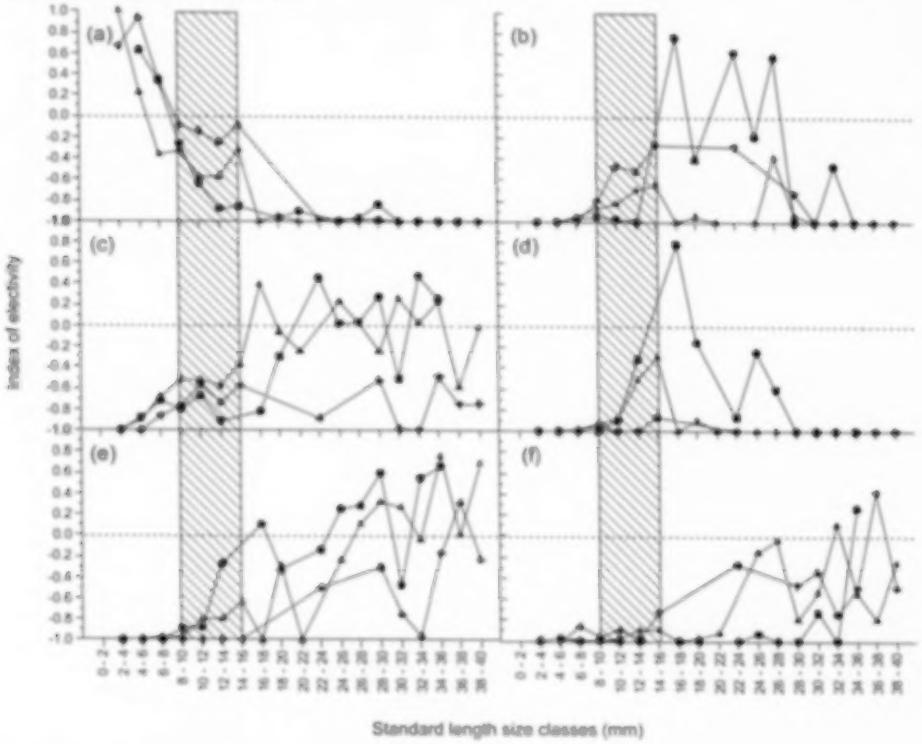
Fig. 4. Relationship between index of electivity (Chesson 1983) and larval fish development in cod (*Gadus morhua*; circles), haddock (*Melanogrammus aeglefinus*; +) and whiting (*Merlangius merlangus*; triangles) for (*a*) nauplii, (*b*) *Oithona similis*, (*c*) *Pseudo/Paracalanus*, (*d*) *Acartia clausii*, (*e*) *Calanus* spp., and (*f*) *Temora longicornis*. Hatched area represents the "intermediate" period of metamorphosis, and the broken line represents the point of no preference.



found similarities in the diets of gadoid larvae in the western Atlantic, in the Irish Sea, the selectivity for different prey species varied between fish species and developmental stages.

In each of the gadoid species studied, nauplii were the only prey item positively selected for in the larval stage, which resulted in high overlap in diets. These high overlaps would suggest that if food was limiting, competition for prey between these gadoid species would be high. However, whiting larvae were seen to differ slightly compared with those of cod and haddock as they had a lower preference for nauplii at the end of the larval stage combined with a higher preference for *Pseudo/Paracalanus*. Last (1978) previously showed that whiting commenced feeding and took larger prey items at a smaller size compared with cod. These observations suggest that whiting could reduce larval competition for food by taking larger prey items at an early age. This could not be due to whiting having a greater gape size as they have been shown to have a smaller gape size at the larval stage than cod and haddock (Rowlands et al. 2006). One possibility could be due to a greater utilization of gape size by whiting. It is known that only a small proportion of the available gape size is used to capture prey (Economou 1991; Sabatés and Saiz 2000; Scharf et al. 2000) and that different larval species can take differently sized prey independent of predator size (Pepin and Penney 1997).

At a standard length of 8 mm, the end of the larval stage was reached and prey selection was observed to change in all gadoids, with a reduction in preference for nauplii along with increased preference for all other copepods. A shift in diet at the end of larval stage was also noted by Thorisson (1989), who observed that cod larvae around Iceland consumed more copepodites than nauplii once they reached a length of 8 mm. An increase in prey size with increasing larval size and development has been noted previously (Last 1978; Robb and Hislop 1980; Sabatés and Saiz 2000). However, it is the observed change at the period of metamorphosis that is important. During this period, individuals will undergo physiological and morphological changes (Kjørsvik et al. 1991; Osse and van den Boogaart 1995; Fuiman and Higgs 1997) requiring higher energetic inputs. Therefore, the selection of diet is particularly important, as there may be an increased risk of starvation (Jordaan and Brown 2003). Differences in prey preference between the gadoid species became more apparent at this period of metamorphosis, as shown by a reduction in diet overlap between species. In particular, the diets of cod and whiting began to diverge from that of haddock. Cod and whiting showed an increased preference for *A. clausii* and *Calanus* spp., but haddock showed little change in diet preference except for an increased selectivity for *O. similis*. However, haddock still showed a high preference for nauplii compared with either cod or whiting, which may indicate potential diet resource partitioning. This selection of small-sized prey items by had-

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dock has been shown previously (Economou 1991) and could be a method of reducing interspecific competition if food were limited (Robb and Hislop 1980). Conversely, diet overlap could increase between developmental stages, as was shown by the high overlap between the larval and intermediate stages of haddock. However, as the intermediate stage individuals are better equipped at locating, consuming, and digesting prey items than larvae (Hunter 1980; Fuiman and Higgs 1997), they would have a greater prey capture success. This could be to the detriment of larval stages from later cohorts in areas of low nauplii abundance as they would not be able to successfully compete for prey if food was limited.

At the juvenile stage, the overlap between cod and whiting (~14 mm) reached its maximum level (~55%), greater than during both the larval and intermediate stages, suggesting increased competition for food. This was caused by cod and whiting larvae exhibiting similar preference throughout juvenile development for the two most dominant species in their diets, *Pseudo/Paracalanus* and *Calanus* spp. Competition between these species would be expected to be high; however, an increase in prey niche size is often expected to increase with larval development (Scharf et al. 2000), which could lead to reduced diet overlap. The observation of increased prey size with development would lead to reduced intraspecific competition for food between early and late-spawned members of a year class (Robb and Hislop 1980).

At the juvenile stage, diet overlap between haddock and cod and haddock and whiting was at its minimum (<25%). The low overlap index observed for haddock diets was explained by the low positive preference shown by haddock for any particular prey species. A lack of diet selectivity in haddock has been noted previously (Robb and Hislop 1980; Kane 1984; Economou 1991). Haddock larvae were shown to take a wider variety of prey items, a feeding strategy that could be beneficial in reducing competition for food. The combination of this lack of preference leading to low overlap between the intermediate and juvenile stages and the suggestion that intermediate haddock could obtain nauplii easier in comparison with smaller larvae suggest that haddock in the intermediate stage would have reduced competition for food compared with cod and whiting at the same developmental stage.

One unifying fact shown by all the gadoids studied was their strong negative preference for *M. lucens* (~3 mm), a large calanoid copepod comparable in size to the highly selected *Calanus* spp. (~4 mm) and occasionally being relatively abundant (see Rowlands et al. 2004). This observation would suggest that prey could be identified and avoided rather than selected purely by size.

Cod and whiting larvae showed a stronger preference earlier in development for adult *Calanus* spp. compared with haddock. However, haddock populations have been observed to fluctuate in accordance with *Calanus* spp. abundances (Cushing 1984; Nash and Geffen 2004; L.J. Buckley, Graduate School of Oceanography, University of Rhode Island/NOAA CMER Program, Narragansett, RI 02882, USA, personal communication). An example of such a fluctuation occurred in 1996 when an increase in *C. finmarchicus* abundance coincided with a strong haddock year class (Nash and Geffen 2004). However, many other copepods were high in abundance in the Irish Sea that year, e.g., *A. clausii*, *Pseudo/Paracalanus*, and *T. longicorni* (M. Edwards, Sir Alistair Hardy Foundation for Ocean Science (SAHFOS), The Laboratory, Citadel Hill, Plymouth PL1 2PB, UK, unpublished data; R.D.M. Nash, unpublished data). Therefore, it is difficult to determine if the occurrence of *Calanus* species is a causative factor in the increase in haddock abundance or if that was due to the general increase in nauplii abundance associated with a year of high copepod abundance. Haddock may be more dependent on a high abundance of nauplii because of their prey preferences throughout, and this could be one possible mechanism influencing the large fluctuations in haddock recruitment (Dickey-Collas et al. 2003).

The calculations of prey selection and thus diet overlap always depend on the abundance and availability of prey items to the larvae (Munk 1995). Pepin and Penney (2000) showed that feeding by larval fish did not exert any significant grazing pressure on zooplankton off the Newfoundland coast. However, Lee et al. (2006) did find that fish larvae varied in condition between years in the Irish Sea, and Thompson and Harrop (1991) suggested that food may be occasionally limiting for fish larvae, although their evidence for this suggestion was weak. Food limitation, if found, would suggest that slight differences in larval feeding strategies could be highly beneficial for the survival of fish larvae. However, the lack of specific predatory niches between cod and whiting does suggest that food is not limiting during these life stages.

In summary, cod and whiting larvae prefer and selectively prey on the same prey species throughout larval development. However, the diet of haddock differs by its preference for nauplii late into metamorphosis. This may be a contributing factor to the large fluctuations in haddock populations (Dickey-Collas et al. 2003), flourishing in years of high nauplii abundances and doing poorly in years of low prey abundance. Even though *Calanus* spp. is of reduced abundance in the Irish Sea (Nash and Geffen 2004), it was preferentially selected by both cod and whiting. This preference was unlikely to be size-related as the similarly sized *M. lucens* was selected against, suggesting that *Calanus* spp. are of great importance in the diets of these gadoids and therefore leaving these gadoids vulnerable to its high interannual spatial and temporal variability (Gowen et al. 1997).

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Population structure of striped marlin (*Kajikia audax*) in the Pacific Ocean based on analysis of microsatellite and mitochondrial DNA

Jan R. McDowell and John E. Graves

Abstract: Genetic variation was surveyed at five microsatellite loci and the mitochondrial control region (819 bp) to test for the presence of genetic stock structure in striped marlin (*Kajikia audax*) collections taken from seven locations throughout the Pacific Ocean. Temporal replicates separated by 9 years were taken off Japan, and three temporal samples spanning 11 years were collected off the coast of eastern Australia. Analyses of multilocus microsatellite genotypes and mitochondrial control region sequences showed no significant heterogeneity among collections taken from the same location in different years; however, significant spatial genetic heterogeneity was observed across all samples for microsatellite markers (F_{ST} = 0.013, $P < 0.001$). Mitochondrial control region sequences were not different across all samples (Φ_{ST} = –0.01, P = 0.642). Analyses of molecular variance (AMOVA) revealed significant genetic differentiation between geographic regions for both microsatellite and mitochondrial markers. These results suggest the presence of genetically discrete populations within the Pacific Ocean and are supported by both the results of tagging studies, which show limited dispersal, and the presence of geographically separated spawning grounds.

Résumé : Nous avons analysé la variation génétique à cinq locus microsatellites et dans la région mitochondriale de contrôle (819 pb) pour vérifier la présence de structure génétique dans des échantillons de stocks de marlins rayés (*Kajikia audax*) provenant de sept sites répartis dans tout le Pacifique. Nous avons pris des échantillons temporels à un intervalle de 9 années au large du Japon et trois échantillons temporels sur une période de 11 ans au large de la côte de l'Australie orientale. Des analyses des génotypes à locus microsatellites multiples et des séquences de la région mitochondriale de contrôle n'indiquent aucune hétérogénéité significative entre les récoltes prises au même site durant des années différentes; on observe, cependant, une hétérogénéité génétique spatiale significative parmi l'ensemble des échantillons chez les marqueurs microsatellites (F_{ST} = 0,013, $P < 0,001$). Les séquences des régions mitochondriales de contrôle ne diffèrent pas dans l'ensemble des échantillons (Φ_{ST} = –0,01, P = 0,642). Des analyses de la variance moléculaire (AMOVA) montrent une différenciation génétique significative entre les régions géographiques, tant chez les marqueurs microsatellites que mitochondriaux. Ces résultats font croire à l'existence de populations génétiquement distinctes au sein du Pacifique, ce qui est corroboré à la fois par les résultats des études de marquage qui révèlent une dispersion limitée et par la présence de sites de fraie séparés géographiquement.

[Traduit par la Rédaction]

Introduction

The factors leading to population structure in large pelagic species, such as istiophorid billfishes, have been subject to much speculation. Unlike less mobile organisms, pelagic species have a vast potential for dispersal and a lack of apparent physical barriers (Graves 1998). In theory, these factors are thought to preclude the development of population genetic structure (Waples 1998; Smedbol et al. 2002). In addition, information that has traditionally been used to appraise the presence of distinct stocks in other marine fishes, such as where and when they spawn, preferred migratory routes, and what comprises suitable habitat, is limited for istiophorid billfishes, making it difficult to assess population structure (Graves and McDowell 2003).

Members of the Istiophoridae, which include the marlins, spearfishes, and sailfish (*Istiophorus platypterus*), generally exhibit limited genetic structure between geographically disjunct samples. The black marlin (*Istiompax indica*) showed no evidence of spatially structured populations between samples collected from throughout the Indo-Pacific (Australia, Vietnam, South Africa, Taiwan, and the eastern Pacific) using either five tetranucleotide microsatellites or restriction fragment length polymorphism (RFLP) analysis of the mitochondrial control region (Falterman 1999). Similarly, the blue marlin (*Makaira nigricans*) showed no evidence of population structure within either the Atlantic or Indo-Pacific oceans based on either nuclear (allozymes, anonymous single copy nuclear DNA), or mitochondrial DNA markers (Graves and McDowell 1995, 2003; Buonaccorsi et al. 1999). However, there were distinct differences between collections of blue marlin from different ocean basins resulting from differences in the distribution of two evolutionarily distinct mitochondrial lineages (Finnerty and

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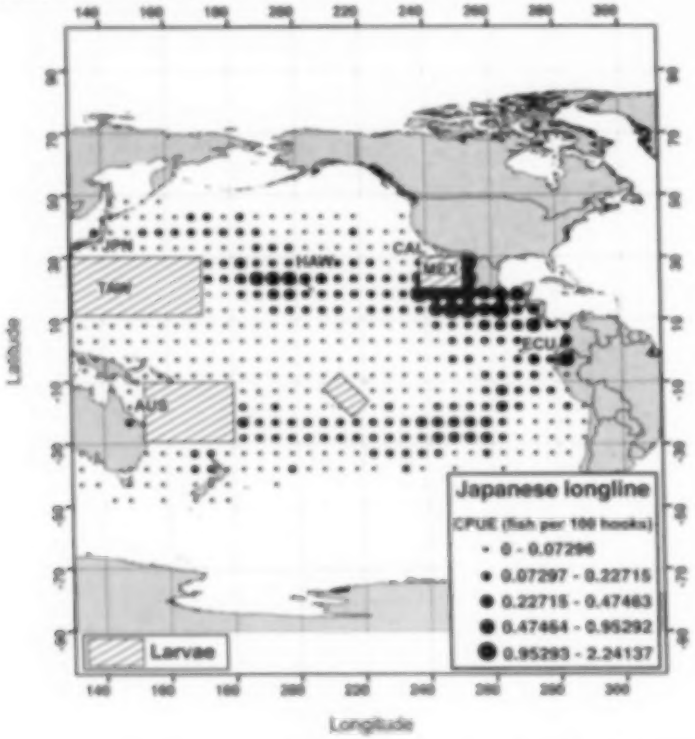
Fig. 1. Distribution of striped marlin (*Kajikia audax*) in the Pacific Ocean as indicated by mean annual Japanese longline catch per unit effort (CPUE) for the period 1970–2000. Hatched boxes indicate identified spawning grounds based on the presence of larvae (reproduced from Bromhead et al. 2004). Collection locations are noted.



Block 1992; Graves and McDowell 1995; Buonaccorsi et al. 2001). Within the *Kajikia*, whole molecule mtDNA RFLP analysis of the white marlin (*Kajikia albidus*) showed no evidence of population structure in the Atlantic among samples taken from the US east coast, the Caribbean, Brazil, and Morocco (Graves and McDowell 2001, 2006).

The striped marlin (*Kajikia audax*) is the most widely distributed (latitudinally) of the billfish species, occurring throughout the tropical, subtropical, and temperate waters of the Pacific and Indian oceans (Nakamura 1985), with occasional observations reported from the Atlantic Ocean near the Cape of Good Hope (Talbot and Penrith 1962; Penrith and Cram 1974). In the Pacific Ocean, striped marlin is distributed between about 45°N and 45°S. Fishery data suggests a horseshoe-shaped distribution across the central North and central South Pacific, with a continuous distribution along the west coast of Central America (Nakamura 1985; Fig. 1). Abundances of striped marlin are highest in the central North Pacific and the eastern tropical Pacific as compared with the southern and western Pacific based on longline data (Ueyanagi and Wares 1975). Pacific striped marlin generally occur between the 20 and 25 °C degree surface isotherms and prefer more temperate waters than other billfishes, which are tropically and subtropically distributed (Nakamura 1985). Striped marlin larvae are primarily found during the late spring and early summer in both hemispheres of the Pacific (Nakamura 1983) and occur in four spatially discrete regions: the eastern North Pacific, the eastern South Pacific, the western North Pacific, and the central South Pacific, suggesting the potential for spawning site fidelity (Fig. 1).

Striped marlin represent an important commercial and recreational resource throughout its range, with the largest catches taken as bycatch by the pelagic longline fisheries targeting tunas (*Thunnus* spp.) (Hinton and Maunder 2003). It has been estimated that 90% of the global commercial catch of billfishes are taken by fisheries targeting other species (King 1990). Pacific-wide landings of striped marlin average around 12 000 t and represent 86% of world landings (Southwest Fisheries Science Center, La Jolla, California). The status of striped marlin as a bycatch species, despite its importance as a recreational species, has resulted in a lack of basic information necessary for effective fishery management, including knowledge of the number of independent stocks and their boundaries (Hinton and Maunder 2003). The inability of traditional fisheries data to resolve the population structure of striped marlin has hindered accurate assessment of the status of striped marlin stocks (Skillman 1990).

In contrast with other billfish species, previous genetic studies of striped marlin have indicated heterogeneity among Pacific striped marlin collections. Allozyme analysis found slight but statistically significant differences among striped marlin samples from Mexico, Ecuador, Australia, and Hawaii in the Pacific (Morgan 1992). Similarly, in an analysis of whole molecule mitochondrial DNA based on RFLP anal-

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ysis using 11 restriction endonucleases from the same samples, several mtDNA haplotypes were present at elevated frequencies within the geographic regions when samples were grouped into eastern, western, and central Pacific regions, while other haplotypes appeared to differentiate individual collections within geographic regions (Graves and McDowell 1994). However, as most haplotypes differed by less than three restriction site changes, net nucleotide sequence divergences (π) between collections were very low (0.01%–0.06%). One major limitation of these studies was the absence of temporal replicates, making the significance of observed differences difficult to evaluate.

The results of previous genetic studies combined with the presence of spatially distinct spawning grounds and the results of tagging studies that suggesting limited dispersal (summarized in Ortiz et al. 2003) have led researchers to postulate that striped marlin do not comprise a single stock in the Pacific Ocean. Furthermore, the lack of large-scale annual movement of large numbers of striped marlin has led to the assertion that striped marlin are much less migratory than previously thought, suggesting that striped marlin are not strictly "highly migratory" like other marlin and tunas (summarized in Bromhead et al. 2004). If the presence of four spatially discrete spawning locations in the Pacific Ocean are driven by (or are a result of) the existence of separate stocks, genetic analysis of striped marlin taken from throughout their range should reveal the presence at least four distinct genetic stocks and confirm the results of earlier studies suggesting spatially structured populations. To test this hypothesis, 373 striped marlin from seven collection locations were screened for genetic variability at five microsatellite loci, and a subset of 85 individuals were sequenced at the mitochondrial DNA control region. In addition, temporal stability of genetic variation was evaluated in collections of striped marlin from Australia and Japan.

Materials and methods

Biological materials

Striped marlin samples were collected from commercial, artisanal, and recreational fisheries at seven sites throughout the Pacific Ocean over a 14-year period ($n = 373$). Locations included Taiwan (TAW) and Japan (JPN) in the northwest Pacific; Port Stephens, Australia (AUS) in the southwest Pacific; Kona, Hawaii (HAW) in the central North Pacific; Cabo San Lucas, Mexico (MEX) and San Diego, California (CAL) in the northeast Pacific; and Manta, Ecuador (ECU) in the southeast Pacific. Replicate samples were collected from JPN in 1994 and 2003 and from AUS in 1994, 1996, and 2005 (Fig. 1, Table 1).

DNA isolation

Samples consisted of either heart tissue removed after capture and stored at –80 °C until isolation or white muscle preserved in 0.25 mmol·L^{-1} ethylenediaminetetraacetic acid (EDTA) pH 8.0, 20% dimethyl sulfoxide (DMSO), and saturated NaCl (Seutin et al. 1991) at room temperature until isolation. DNA was isolated using either a phenol–chloroform (Sambrook and Russell. 2001) or a proteinase K – chelex extraction (Estoup et al. 1996).

Microsatellites

Five microsatellite loci originally developed for use in the blue marlin (*Mn01*, *Mn08*, *Mn10*, *Mn60*, and *Mn90*) were amplified using the parameters outlined in Buonaccorsi and Graves (2000). Microsatellite loci were analyzed on a LI-COR 4200 Global IR^2 automated sequencer (LI-COR Inc., Lincoln, Nebraska, USA). A 50–350 base pair (bp) size standard (LI-COR) was loaded onto each end and at the center of each gel to determine allele sizes. To ensure identical scoring of alleles at a locus across gels, between four and eight lanes of each run consisted of samples for which allele sizes were known. Alleles sizes were measured using the Gene ImagIR 4.03 software (LI-COR). Approximately 20% of the samples were measured twice to verify that alleles could be consistently scored.

The MICRO-CHECKER 2.2.1 software (van Oosterhout et al. 2004) was used to identify possible genotyping errors such as stuttering, large allele dropout, and null alleles within the microsatellite data set by performing 10 000 randomizations. GENEPOP 3.1b (Raymond and Rousset 1995) was used to perform exact tests (10 000 iterations; Guo and Thompson 1992) for deviations of genotypic distributions from the expectations of Hardy–Weinberg equilibrium and to calculate observed (H_O) and expected (H_E) heterozygosities and test for heterozygote excess and deficiencies in the microsatellite data. The program FSTAT 2.9.3 (Goudet 1995) was used to estimate allelic richness per locus and sample (R_s) to allow comparison of the number of alleles independent of samples size for microsatellite data.

The ARLEQUIN software package (Excoffier et al. 2005) was used to estimate Weir and Cockerham's (1984) unbiased estimator of Wright's F statistics (F_{ST}) and for hierarchical F_{ST} analyses (analysis of molecular variance, AMOVA, 10 100 permutations) on microsatellite data. A principal component analysis (PCA) was performed on the multilocus microsatellite data using the program PCAGEN (J. Goudet, Institute of Ecology, Université de Lausanne, CH-1015, Lausanne, Switzerland, www2.unil.ch/popgen/softwares/pcagen.htm). Populations were ordinated according to the first and second axes. The percent inertia of each PCA axis and its P value were assessed by performing 10 000 randomizations of genotypes.

The program MIGRATE (Beerli and Felsenstein 2001; Beerli 2002) was used to estimate both theta (Θ), which is equal to $4N_e\mu$ (where N_e is the long-term (inbreeding) effective population size, and μ is the mutation rate) and M, which is equal to the ratio m/μ (where m is the immigration rate). Analyses were performed under the allele model, as not all microsatellite loci conformed to the strict expectations of the stepwise mutation model (i.e., there were missing steps in the distribution of alleles for some loci). Each Markov chain Monte Carlo (MCMC) run consisted of 10 short chains (sampling 10 000 trees) and three long chains (sampling 100 000 trees) with a burn-in period of 10 000 trees. All runs were repeated five times to verify consistency of results, and the data presented are the average of the five runs.

Control region

Subsets of 12 individuals from each of the sampling locations were sequenced for an 819 bp fragment of the mito-

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Table 1. Summary statistics for five microsatellite loci among striped marlin (*Kajikia audax*) samples.

	Locus					
Sample	*Mn01*	*Mn08*	*Mn10*	*Mn60*	*Mn90*	Average across loci
AUS 1994						
n	37	37	37	37	37	
a	9	20	12	23	25	
R_s	7.93	17.25	11.08	19.54	21.50	15.46
RPT range	14–22	7–35	7–24	12–38	22–88	
H_E	0.77	0.93	0.89	0.94	0.95	0.896
H_O	0.78	0.92	0.94	0.95	0.97	0.912
HW	0.359	0.323	0.392	0.972	0.979	
AUS 1996						
n	27	27	27	27	27	
a	8	19	9	18	22	
R_s	7.88	18.49	8.99	17.18	21.15	14.74
RPT range	14–22	7–33	11–21	14–37	46–90	
H_E	0.81	0.94	0.84	0.93	0.95	0.894
H_O	0.67	0.96	0.78	0.93	0.96	0.860
HW	0.589	0.729	0.383	0.383	0.938	
AUS 2005						
n	33	33	33	33	33	
a	11	20	14	20	26	
R_s	10.2	18.89	12.54	18.55	24.16	16.87
RPT range	14–35	7–32	7–21	12–41	42–88	
H_E	0.84	0.87	0.87	0.95	0.84	0.874
H_O	0.91	0.88	0.85	0.85	0.82	0.862
HW	0.711	0.868	0.427	0.200	0.664	
CAL 2000						
n	39	39	39	39	39	
a	9	22	14	23	24	
R_s	8.60	18.33	12.09	20.51	19.91	15.89
RPT range	14–22	10–35	7–22	12–52	22–90	
H_E	0.853	0.937	0.884	0.952	0.938	0.913
H_O	0.795	0.923	0.897	0.923	0.923	0.982
HW	0.326	0.447	0.878	0.550	0.186	
ECU 1995						
n	39	39	39	39	39	
a	8	22	15	25	22	
R_s	7.73	18.50	12.44	20.06	18.60	15.46
RPT range	14–21	10–38	7–24	14–51	46–90	
H_E	0.775	0.935	0.871	0.941	0.941	0.893
H_O	0.795	0.949	0.846	0.974	0.974	0.908
HW	0.471	0.331	**0.004**	0.682	0.864	
HAW 1998						
n	48	48	48	48	48	
a	10	23	14	27	23	
R_s	8.19	18.84	12.60	20.97	19.00	15.92
RPT range	14–39	3–37	7–22	12–53	30–90	
H_E	0.837	0.947	0.891	0.955	0.931	0.745
H_O	0.75	0.937	0.937	0.875	0.771	0.854
HW	0.110	0.325	0.503	0.380	0.186	

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Table 1 (*concluded*).

Sample	Locus					Average across loci
	Mn01	*Mn08*	*Mn10*	*Mn60*	*Mn90*	
MEX 1994						
n	32	32	32	32	32	
a	7	20	11	18	20	
R_s	6.98	18.43	9.90	16.95	17.84	14.02
RPT range	15–21	10–38	7–21	14–55	52–86	
H_E	0.83	0.936	0.734	0.936	0.935	0.874
H_O	0.781	0.906	0.844	0.937	0.969	0.887
HW	0.140	0.296	0.252	0.478	0.315	
TAW 1998						
n	24	24	24	24	24	
a	8	17	12	14	19	
R_s	8	17	12	14	19	14
RPT range	10–20	12–34	7–22	14–33	46–90	
H_E	0.825	0.941	0.891	0.906	0.945	0.902
H_O	0.667	0.958	0.875	0.792	0.917	0.842
HW	0.106	0.983	0.447	0.191	0.555	
JPN 1994						
n	39	39	39	39	39	
a	11	26	18	24	28	
R_s	10.00	19.44	11.90	18.88	18.05	15.67
RPT range	10–22	7–34	7–22	12–38	45–88	
H_E	0.864	0.945	0.881	0.942	0.935	0.913
H_O	0.795	1	0.897	0.974	0.923	0.918
HW	0.176	0.976	0.056	0.128	0.848	
JPN 2003						
n	53	53	53	53	53	
a	9	26	17	22	25	
R_s	8.04	20.50	13.94	18.65	19.21	16.07
RPT range	10–21	7–36	7–31	12–35	46–94	
H_E	0.801	0.952	0.89	0.943	0.943	0.906
H_O	0.623	0.943	0.849	0.887	0.962	0.853
HW	**0.006**	0.887	0.053	**0.005**	0.957	

Note: AUS, Australia; CAL, California; ECU, Ecuador; HAW, Hawaii; MEX, Mexico; TAW, Taiwan; JPN, Japan; *n*, number of individuals; *a*, number of alleles; R_s, allelic richness per locus and sample; RPT range, size range in number of repeats; H_E, expected heterozygosity; H_O, observed heterozygosity; HW, probability of concordance with Hardy–Weinberg expectations. Values in bold are significant after correction for multiple tests (initial $\alpha = 0.05/5 = 0.01$).

chondrial control region, with the exception of individuals from the CAL sample, which failed to amplify (85 individuals were sequenced, GenBank accession Numbers DQ199950–DQ200028). Samples were amplified using the Pro-5′ and 12SAR-3′ primers (Palumbi et al. 1996; CACGACGTTGTAAAACGACCTACCYCYAACTCCCAAAGC and GGATAACAATTTCACACAGGGCATAGTGGGGTATCTAATCC, respectively). Primers were modified to include M13 tails. Amplified products were sequenced on a LI-COR 4200 Global IR^2 system using IRD-800-labelled forward primer and IRD-700-labelled reverse primer (LI-COR). M13F (–29) and an internal reverse primer designed specifically for the teleost control region (CCATCTTAACATCTTCAGTG; S. Boles, Virginia Institute of Marine Science, Rt 1208 Greate Road, Gloucester Point, VA 23062, USA, unpublished data) were used for sequencing. Standard chromatographic curves of forward and reverse sequences were imported into the program Sequencher 4.2.2 (Gene Codes Corp., Ann Arbor, Michigan, USA), aligned, and edited. A consensus of forward and reverse sequences was created and exported to the program MacVector 7.2.3 (Oxford Molecular LTD, Madison, Wisconsin, USA), and an alignment was created using the CLUSTAL W algorithm (Thompson et al. 1994) and adjusted by eye.

The ARLEQUIN software package (Excoffier et al. 2005) was used to estimate Φ_{ST} (an mtDNA analogue for F_{ST}; Excoffier et al. 1992) and for hierarchical Φ_{ST} analyses on control region sequences as described above. The Φ_{ST} analyses were performed using a matrix of Tamura and Nei (1993) distances. The program ARLEQUIN (Excoffier et al. 2005) was also used to calculate haplotype diversity (h) and nucleotide diversity (π) for the mtDNA control region se-

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quence data. PAUP* 4.0 (Swofford 2000) was used to generate the table of variable sites and a neighbor-joining (Saitou and Nei 1987) tree based on the Tamura–Nei distance (Tamura and Nei 1993; Fig. 2). DnaSP 4.0 (Rozas et al. 2003) was used to estimate the nearest-neighbor statistic, S_{nn} (Hudson 2000), for the mtDNA control region sequences using 10 000 permutations and gaps excluded in pairwise comparisons. The S_{nn} statistic measures how often the nearest neighbors in sequence space are from the same locality in geographical space and is particularly appropriate when h is large and sample sizes are small (Hudson 2000). Significance levels were adjusted using the sequential Bonferroni technique (Rice 1989) in all cases of multiple tests.

Results

Genetic variation

For microsatellite data, the number of alleles per locus varied from 7 at locus *Mn01* to 27 at locus *Mn60* (Table 1). Allelic richness per locus and sample ranged from 6.98 at locus *Mn01* in the MEX 1994 sample to 24.16 in the AUS 2005 sample at locus *Mn90*; the average across loci ranged from 14 in TAW 1998 to 16.87 in AUS 2005 (Table 1). Average observed heterozygosities ranged from 0.67 at locus *Mn01* in the AUS 1996 and TAW 1998 samples to 0.97 at the *Mn60* (ECU 1995 and JPN 1994) and *Mn90* (AUS 1994, ECU 1995, MEX 1994) loci; the average across loci ranged from 0.84 in TAW 1998 to 0.913 in CAL 2000 (Table 1). Average expected heterozygosities ranged from 0.73 in the MEX 1994 sample at *Mn10* to 0.96 in the HAW 1998 sample at *Mn60* and from 0.74 for HAW 1998 to 0.91 for CAL 2000 and JPN 1994 (Table 1). Three of the 50 tests for conformance to the expectations to Hardy–Weinberg equilibrium differed significantly after corrections for multiple tests. The ECU 1995 sample had a genotypic distribution that differed significantly at *Mn10*, and the JPN 2003 sample differed at *Mn01* and *Mn60* (heterozygote deficits; Table 1). Analysis with the MICRO-CHECKER software showed no evidence that null alleles, stuttering, or large allele dropout affected any of the loci, and rescoring of samples confirmed that alleles were being sized consistently across gels; consequently, all loci were included in all analyses.

Aligned mitochondrial control region sequences ranged from 781 to 807 bp, excluding gaps. Overall, 570 characters were constant, 249 were variable, and 58 insertions–deletions were observed. Of the 249 variable sites, 162 were parsimony informative. For mtDNA, there were 79 haplotypes detected among the 83 striped marlin sequenced at the control region, and no haplotype was detected more than twice. Among the four haplotypes that were shared, one was common to two JPN samples, one occurred in JPN and TAW, one in JPN and HAW, and one in HAW and MEX (Fig. 2). Haplotype diversity (h) was 0.998 over all samples and ranged from 0.98 to 1.0. Nucleotide diversity (π) was 0.044 ± 0.021 over all samples and ranged from 0.039 ± 0021 in the AUS sample to 0.054 ± 0.029 in the TAW sample.

Population structure based on microsatellites

The presence of temporal stability of the AUS (1994, 1996, 2005) and JPN (1994, 2003) samples was assessed using pairwise multilocus F_{ST} values (10 000 permutations of the data). In addition, an AMOVA was conducted for the three AUS samples. The F_{ST} between the two JPN samples was –0.0001 (P = 0.540). F_{ST} values for the three Australian samples ranged from –0.0035 (P = 0.872) between AUS 1994 and AUS 2005 to 0.0047 (P = 0.093) between AUS 1996 and AUS 2005; no values were significant. An AMOVA among the AUS collections indicated that only 0.12% of the variation was among samples (P = 0.252, Table 2) while 99.88% was within samples.

To examine whether results of multilocus F_{ST} analyses were being driven by a single locus, population pairwise F_{ST} values were calculated separately for each locus. In population pairwise F_{ST} comparisons across all loci, 18/21 (85.7%) were significant ($P < 0.05$, data not shown), and results for individual loci ranged from 8/21 (38%) for *Mn60* to 14/21 (66.7%) for *Mn01*. The global multilocus F_{ST} across all samples was 0.013 ($P < 0.001$), and single locus values ranged from 0.0336 at *Mn01* to 0.0067 at *Mn90*; all values were highly significant ($P < 0.001$).

Since there was no evidence of temporal variation among samples in the microsatellite data, the temporal replicate collections were pooled for subsequent analyses. The global F_{ST} among all samples was 0.0134 ($P < 0.001$). Pairwise multilocus F_{ST} values ranged from 0.0004 (P = 0.435) between CAL and JPN to 0.0276 ($P < 0.001$) between MEX and AUS; in general, F_{ST} values were highest between the MEX sample and all other locations (Table 3). AMOVAs were performed to maximize the amount of variance due to variation among groups of samples (regions). Since the JPN, HAW, and CAL samples were not significantly different in pairwise comparisons (Table 3), they were grouped together for AMOVA analysis and collectively designated "northern" resulting in an F_{ST} of 0.002 (P = 0.089). Addition of TAW to the northern sample resulted in an F_{ST} of 0.004 (P = 0.005), a small but significant difference among collections within the group. Grouping samples into strictly northern (JPN, TAW, HAW, CAL) and southern (AUS, ECU, MEX) groups revealed small but significant spatial differences among regions (F_{CT} = 0.004, $P < 0.001$). However, grouping samples into strictly east (JPN, TAW, AUS) and west (CAL, MEX, ECU) collections revealed no significant spatial differentiation among the samples (F_{CT} = –0.002, P = 0.696). Differences among regions (% variation and fixation index) were maximized and variation within regions was minimized when the northern collection was grouped and all remaining collections (AUS, MEX, ECU) were held separately (F_{CT} = 0.014, $P < 0.0001$). Likewise, the PCA analysis (Fig. 3) indicated that the JPN, TAW, CAL, and HAW composed a single (northern) group, while the AUS, MEX, and ECU collections appeared to represent distinct groups. The two principal axes together explained 50.67% of the total genetic diversity. The first principal component explained 29.81% ($P < 0.001$) of the variance among the populations, and the second axis accounted for 20.86% of the variance ($P < 0.001$). The third component accounted for 11.84% of the variance and was not significant (P = 0.989).

Population structure based on mtDNA

Unlike results from the microsatellite markers, there were

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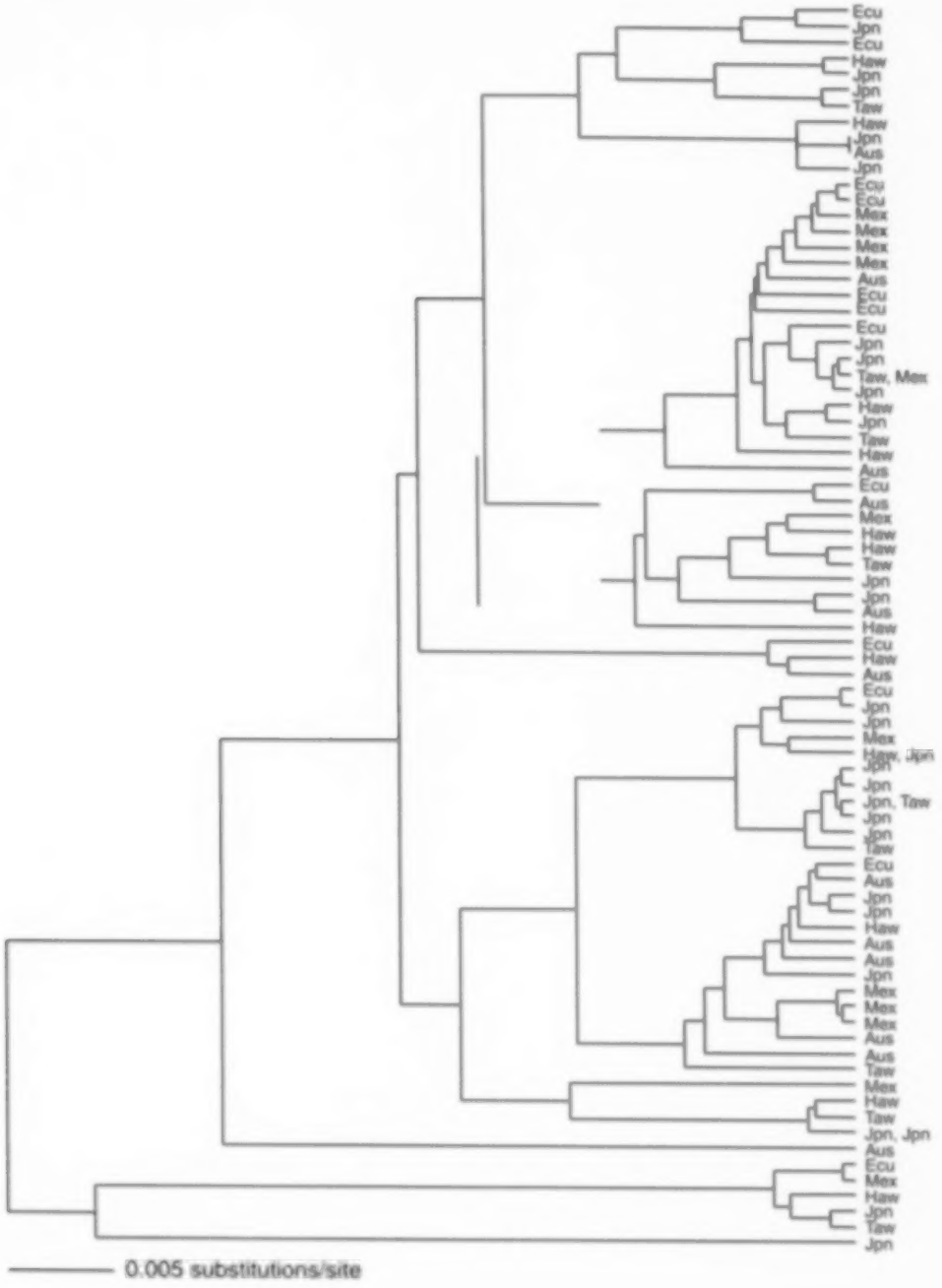
Fig. 2. Neighbour-joining tree of striped marlin (*Kajikia audax*) mitochondrial control region sequences (819 bp) based on the Tamura and Nei (1993) distances. Sample designations are as follows: Japan, Jpn; Taiwan, Taw; Australia, Aus; Hawaii, Haw; California, Cal; Mexico, Mex; and Ecuador, Ecu.



no significant pairwise comparisons of Φ_{ST} based on Tamura–Nei distances (Tamura and Nei 1993). Values ranged from –0.0587 between HAW and TAW to 0.0430 between ECU and AUS. However, the nearest-neighbor statistic (S_{nn}) revealed a significant, nonrandom association between mtDNA sequence similarity and geographic location ($S_{nn} = 0.202$, $P < 0.0001$). An AMOVA indicated that population divisions were essentially the same as those obtained from microsatellite data. Grouping the JPN, TAW, and HAW collections into the northern group and holding all

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Table 2. Analysis of molecular variance (AMOVA) among regions and locations for striped marlin (*Kajikia audax*) based on the number of alleles (F_{ST}) for microsatellite data.

	Source of variation (F_{ST})	% variation	Fixation index	*P*
Samples				
All samples	Among samples	1.35	0.013	<0.0001
	Within samples	98.65		
Temporal stability	Among samples	0.12	0.001	0.252
(AUS 1994, 1996, 2005)	Within samples	99.88		
Temporal stability	Among samples	–0.01	0.000	0.543
(JPN 1994, 2003)	Within samples	100.01		
North	Among samples	0.20	0.002	0.089
(JPN, HAW, CAL)	Within samples	99.80		
North	Among samples	0.40	0.004	0.005
(JPN, TAW, HAW, CAL)	Within samples	99.60		
South	Among samples	2.03	0.020	<0.0001
(AUS, ECU, MEX)	Within samples	97.97		
South	Among samples	2.13	0.021	<0.0001
(AUS, ECU, MEX, TAW)	Within samples	97.87		
North and South	Among regions	0.50	0.004	<0.0001
(North: HAW, JPN, TAW, CAL;	Among populations and regions	1.05	0.011	<0.0001
South: AUS, ECU, MEX)	Within populations	98.45	0.015	<0.0001
North and South	Among regions	0.22	0.002	0.210
(North: HAW, JPN, CAL;	Among populations and regions	1.22	0.012	<0.0001
South: AUS, ECU, MEX, TAW)	Within populations	98.56	0.014	<0.0001
East and West	Among regions	–0.15	–0.002	0.696
(East: JPN, TAW, AUS;	Among populations and regions	1.6	0.016	<0.0001
West: CAL, MEX, ECU)	Within populations	98.55	0.014	<0.0001
Regions				
North; Southwest; Southeast	Among regions	0.70	0.007	0.008
(North: JPN, HAW; Southwest: AUS, TAW;	Among populations and regions	0.82	0.008	<0.0001
Southeast: MEX, ECU)	Within populations	98.48	0.152	<0.0001
North; MEX; ECU; (AUS, TAW)	Among regions	0.75	0.008	0.038
(North: JPN, HAW, CAL)	Among populations and regions	0.76	0.008	<0.0001
	Within populations	98.48	0.015	<0.0001
North; MEX; ECU; AUS	Among regions	1.28	0.012	<0.0001
(North: JPN, HAW, CAL, TAW)	Among populations and regions	0.40	0.004	<0.0001
	Within populations	98.32	0.017	<0.0001

Note: Significance was assessed using 10 100 permutations. See Table 1 for location abbreviations.

other samples separate resulted in a Φ_{CT} = 0.034 (P < 0.0001; Table 4).

Migration and effective population size

The average Θ values estimated with the MIGRATE software for the microsatellite data set ranged from 0.2923 (SE = 0.0277) in AUS to 1.0512 (SE = 0.1258) in JPN (Fig. 4). The estimates of per generation immigration rates were also variable among samples (only values >20 are shown in Fig. 4 to facilitate presentation of data). Analysis of the historical migration rate (M) indicates that gene flow has historically been asymmetrical, and JPN has been the most important contributor to gene flow. For JPN, TAW, and ECU, mutation has likely been more important than gene flow for introducing variation into the population, while gene flow appears to have been more important for bringing new alleles into MEX and CAL. Interestingly, AUS appears to have received immigrants from the North Pacific, but has been an important source of immigrants for CAL and MEX in the eastern Pacific.

Discussion

Stock structure

The results of the AMOVA and PCA analyses clearly delineate the presence of at least four genetically discrete groups of striped marlin within the Pacific Ocean, which correspond to the four described striped marlin spawning areas. As with previous genetic studies, our results show genetic homogeneity between temporal replicates, indicating that the observed genetic heterogeneity among samples from different locations reflects geographical differences in the genetic structure rather than sampling error. For the microsatellite data, a locus-by-locus analysis shows that the observed structure was not driven by any single locus or pair of loci (data not shown). These results are corroborated by

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Table 3. Matrix of pairwise F_{ST} values based on microsatellite data (below diagonal) and pairwise Φ_{ST} values for mtDNA sequences based on the Tamura–Nei distance (Tamura and Nei 1993) (above diagonal).

	AUS	CAL	ECU	HAW	MEX	TAW	JPN
AUS	—	NA	0.0430 (0.775)	0.0184 (0.210)	0.0143 (0.245)	0.0045 (0.324)	0.0167 (0.200)
CAL	0.0156 (<0.001)	—	NA	NA	NA	NA	NA
ECU	0.0174 (<0.001)	0.0104 (<0.001)	—	−0.0300 (0.775)	−0.0130 (0.598)	−0.2740 (0.688)	0.0186 (0.199)
HAW	0.0104 (<0.001)	0.0028 (0.131)	0.0145 (<0.001)	—	0.0225 (0.431)	−0.0587 (0.950)	0.0003 (0.403)
MEX	0.0276 (<0.001)	0.0232 (<0.001)	0.0114 (0.002)	0.0224 (<0.001)	—	−0.0320 (0.669)	0.0260 (0.151)
TAW	0.0231 (<0.001)	0.0082 (0.025)	0.0187 (<0.001)	0.0118 (0.002)	0.0274 (<0.001)	—	−0.0424 (0.151)
JPN	0.0165 (<0.001)	0.0004 (0.435)	0.0100 (<0.001)	0.0030 (0.052)	0.0152 (<0.001)	0.0068 (0.020)	—

Note: Significance values (in parentheses) are based on 10 000 permutations. NA, not available. See Table 1 for location abbreviations.

our observation of population structure based on data from the mtDNA locus and suggest the presence of a minimum of four long-term, temporally stable genetic stocks of striped marlin in the Pacific Ocean.

In the southwest Pacific, our temporal samples spanning 11 years off Port Stephens, Australia, suggest that genetic variation in striped marlin is temporally stable, at least in this area. Pairwise comparisons of the AUS collection with other all collections were highly significant, and the AUS collections were clearly separated from the other collections in the PCA analysis. These results are consistent with other fisheries data. Striped marlin are known to spawn off eastern Australia based on the presence of larvae. In addition, tagging data shows that the majority of striped marlin released off Australia are recaptured within several hundred nautical miles (1 n.m. = 1.852 km) of release even after 6–9 months at liberty and have a mean displacement of 200 n.m., suggesting that striped marlin in this area are residential (Ortiz et al. 2003). Additionally, no tag–recaptures of marlin released in the southwest Pacific have been recorded in the eastern Pacific or vice versa (Ortiz et al. 2003).

Like the southwest Pacific, the ECU collection in the southeast Pacific was significantly different from all other samples based on microsatellite data. Pairwise F_{ST} comparisons between ECU and other samples were all significant, and as with previous studies (Graves and McDowell 1994), ECU was distinct from the eastern Pacific (MEX) collection. Tagging studies have indicated that movements in the southeast Pacific are complex, but that there is a general movement towards the Galapagos and southern Central American coastline, which then expands offshore and southwest in October–March. This has been correlated to the migration of mature individuals towards the spawning grounds (Kume and Joseph 1969), as over 75% of fish caught in this area at this time are mature, and supports the lack of mixing indicated by genetic data.

Several lines of evidence support the genetic clustering of the northern striped marlin samples (JPN, HAW, TAW, and CAL). The striped marlin has a continuous horseshoe-shaped distribution with one arm that extends across the North Pacific. Tag–recapture data have shown movements into the area around Hawaii by predominantly 40–60 kg striped marlin migrating from Californian coastal waters from late autumn – early winter. However, there have been no California recaptures of striped marlin tagged in Hawaii (Ortiz et al. 2003), nor are fish in spawning condition caught off the coast of California. Whether there is movement between the central North Pacific (HAW) and western Pacific (JPN, TAW) is unknown because there has been negligible tagging effort in the northeast Pacific. However, although striped marlin spawn in the region southeast of Japan (Squire and Suzuki 1990), few juveniles are caught in this area and it has been suggested that they may migrate from this region, returning when they become mature (Bromhead et al. 2004). This supposition is supported by the fact that juvenile striped marlin appear in the central North Pacific at around 10 kg, and neither larger fish nor larvae are found in this area, suggesting that juveniles use the central North Pacific as a feeding ground (Matsumoto and Kazama 1974). Taken together, these data imply that juvenile striped marlin spawned off Japan may be moving east into the central

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Fig. 3. Principal component analysis (PCA) showing the genetic relationship among striped marlin (*Kajikia audax*). PCA axis 1 explains 29.81% of the variance; axis 2 explains 20.86% of the variance. Circles are drawn around groups of collections based on results of pairwise F_{ST} values; nonsignificant comparisons are grouped.

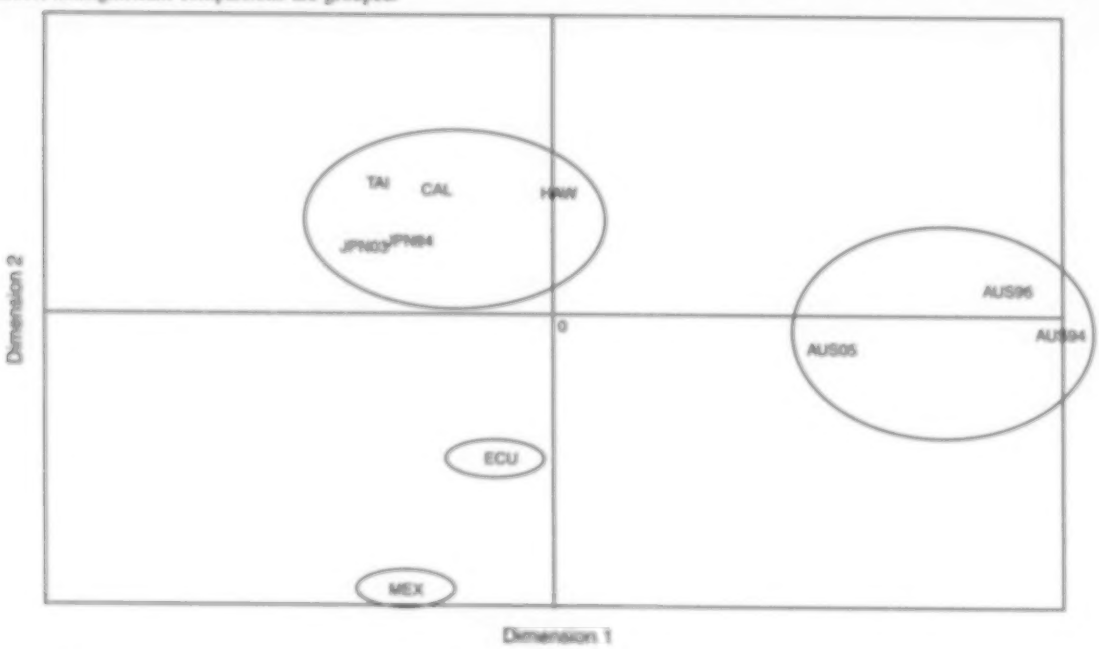

Table 4. Analysis of molecular variance (AMOVA) among regions and locations for striped marlin (*Kajikia audax*) collections based on mtDNA data.

	Source of variation (Φ_{ST})	% variation	Fixation index	*P*
All samples	Among samples	–0.67	–0.007	0.642
	Within samples	100.67	—	—
(JPN, TAW, HAW); (MEX, ECU); AUS	Among regions	3.14	0.031	<0.0001
	Among populations and regions	–2.77	–0.029	0.710
	Within populations	99.63	0.004	0.623
(JPN, HAW, TAW); MEX; ECU; AUS	Among regions	3.40	0.034	<0.0001
	Among populations and regions	–3.12	–0.032	0.377
	Within populations	99.71	0.003	0.633
(JPN, TAW); MEX; ECU; AUS; HAW	Among regions	4.31	0.043	<0.0001
	Among populations and regions	–4.39	–0.046	0.286
	Within populations	100.08	–0.001	0.637
(JPN, TAW); (MEX, ECU); AUS; HAW	Among regions	3.47	0.035	0.005
	Among populations and regions	—	–3.49	–0.036
	Within populations	100.02	–0.000	0.632

Note: Φ_{ST} was calculated based on Tamura–Nei distance (Tamura and Nei 1993). Significance was assessed using 10 100 permutations. The two years of JPN data were held separately in this analysis, although they are represented as a single year below. See Table 1 for location abbreviations.

Pacific to feed and that some of these fish may end up off the coast of California before returning to the spawning grounds off Japan.

Although the TAW samples grouped most closely with the other northern samples and no significant Φ_{ST} differences were noted between TAW and other northern samples based on mtDNA data, microsatellite data revealed small but significant pairwise differences between TAW and CAL and between TAW and JPN. Striped marlin larvae have been found in the eastern Indian Ocean off the northwest coast of Australia (Nakamura 1983) as well as in the Timor and Banda seas (Ueyanagi and Wares 1975). Derivation of the TAW samples from a mixture of both the Indian Ocean and western North Pacific spawning stocks is one possible explanation for the observed differences, and the relationship between Pacific Ocean and Indian Ocean striped marlin should be examined further in future studies.

One of the most interesting results of the current study is

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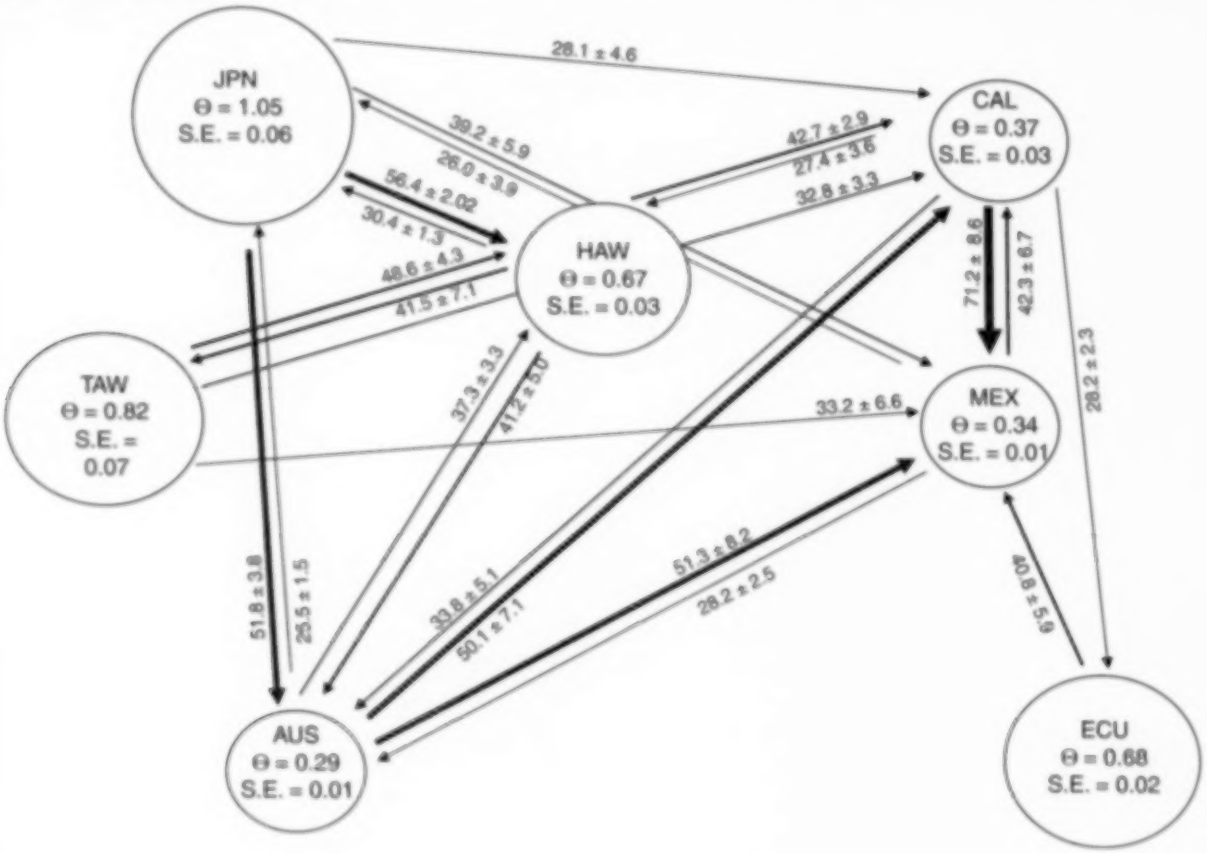
Fig. 4. Historical effective population size (Θ) and migration rate scaled by mutation (*M*) between populations, including the standard errors across the five runs using the program MIGRATE (Beerli and Felsenstein 2001). Values of *M* less than 20 are not shown. See Table 1 for location abbreviations.



the presence of statistically significant differences between striped marlin taken off California (CAL) and those taken off Baja California (MEX), despite the fact that tagging studies clearly demonstrate migration between these areas. Movements of Mexican fish northward towards California during the summer months have been documented (e.g., Armas et al. 1999), and most tagged southern California fish are recaptured off Baja California (Ortiz et al. 2003). In addition, longline data show that fluctuations in catch rates in the Baja California region are correlated with fluctuations in the rest of the eastern Pacific, suggesting considerable mixing within this area (Squire and Au 1990). Although our results could be due to sampling error associated with insufficient sample size, there are data to support our finding. In the Northeast Pacific off Baja California, 65% of fish caught are mature (Squire and Suzuki 1990), while no fish in spawning condition are caught off the coast of California (M. Hinton, Inter-American Tropical Tuna Commission, 8604 La Jolla Shores Drive, La Jolla, California, personal communication, 2006). In addition, most striped marlin recaptured off Baja California are caught within a few hundred nautical miles of release even after nearly 2 years at liberty (Ortiz et al. 2003). Perhaps the most convincing support for validity of results suggesting that the MEX and CAL collections are representative of distinct populations is based on data from satellite tagging. Since 2000, 115 satellite tags have been put on striped marlin near Magdelena Bay, Baja California, Mexico. Although they have been tracked for up to 9 months (November–July), none have been found to range from Mexican waters (M. Domeier, Pfleger Institute of Environmental Research, Oceanside, California, personal communication). Taken together, the available data suggest the presence of a self-sustaining resident population of striped marlin off the coast of Mexico. Since it is thought that striped marlin off the coast of California are composed of subadult fish that are thought to travel to spawning grounds in the western Pacific upon maturity, it seems plausible that striped marlin from both stocks mix on offshore feeding grounds. This mixing could explain the apparent conflict between the genetic and conventional tagging data. Other large pelagic species, most notably the bluefin tuna (*Thunnus thynnus*), have recently been shown to mix on feeding grounds but maintain distinct stocks driven by spawning site fidelity (Carlsson et al. 2006).

Microsatellite data are capable of detecting small genetic

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differences between populations because of their high mutation rate. This has led to the realization that statistically significant differences between populations do not always indicate biologically significant differences (Waples 1998; Hedrick 1999). However, the results of this study correlate well with the other fishery data; the seemingly cyclical movements of adults with season combined with the presence of distinct spawning grounds. In addition, just over 60% of global recaptures of tagged striped marlin occur within 200 n.m. of the release point, and 84% occur within 500 n.m., while less than 8% are recaptured over 1000 n.m. from the point of release, indicating at least some level of regional fidelity (Ortiz et al. 2003; Bromhead et al. 2004). There are also morphological differences between northern and southern Pacific striped marlin; southern fish are larger than northern fish and there are differences in pectoral fin length (Ueyanagi and Wares 1975). Finally, analysis of longline data has shown that catch rates of striped marlin near the equator in the western Pacific are exceptionally low, which further implies the potential for separate northern and southern stocks particularly in the west (Ueyanagi and Wares 1975).

Historical migration and effective population size

Examination of the asymmetric matrix of historical migration rates and relative effective population size estimates suggest historical patterns that are not apparent using traditional genetic analyses. Although no genetic differentiation was noted among TAW, JPN, HAW, and CAL, estimates of long-term effective population sizes (θ) in these samples ranged from 1.05 to 0. 37. This discrepancy may result from sampling different components of the population in the different areas; mature fish are not found in HAW or CAL, and the effective population sizes in these areas may appear smaller owing to the exclusion of some age classes. Alternately, striped marlin may have arisen in the western Pacific and subsequently spread eastward to HAW and CAL, although this might be expected to result in fewer alleles per locus in the HAW and CAL samples, which was not evident in this study. Finally, the larger effective population sizes of the JPN and TAW samples may be due to immigration from other (Indian Ocean) source populations not included in this study, and the more immigrants from the unknown populations that are arriving in the sample populations, the more inflated the estimated population sizes (Beerli 2004).

Interestingly, estimates of θ suggest that the relative long-term inbreeding effective population size of the CAL and MEX samples are nearly identical (0.377 vs. 0.337, respectively) and that of all pairwise comparisons, these two samples have historically had the largest exchange of migrants between them (71 and 43, respectively), suggesting that they comprise two samples from a single population. This corroborates the observed movements of fish between the two areas seen in tagging studies (Armas et al. 1999; Ortiz et al. 2003) but is contrary to the results of both the satellite tagging data (M. Domeier, Pfleger Institute of Environmental Research, Oceanside, California, personal communication) and the finding that these samples are genetically distinct. The discrepancy between the high level of historical exchange and an apparent lack of contemporaneous gene flow is intriguing. If a subset of the samples taken from each location actually belonged to the other stock (some samples were taken at a time and place when stocks were mixed on feeding grounds), the apparent high level of historical migration could simply be an artifact and this seems the most likely scenario. This highlights the need for further study of population structure in striped marlin, as samples used in this study were taken from adult specimens of unknown natal origin rather from juveniles. Recent studies of bluefin tuna (Carlsson et al. 2004) and loggerhead turtles, *Caretta caretta*, (Bowen et al. 2005) have demonstrated that fine-scale population structure may not be fully elucidated by examination of a single life-history stage, and population structure may in fact be obscured by improper sampling.

Results of our study suggest that striped marlin comprises multiple genetic stocks. Northern collections of striped marlin taken from Japan, Taiwan, Hawaii, and California appear to constitute a single genetic stock, while southern collections from Australia, Mexico, and Ecuador are statistically significantly different from each other as well as from the northern samples. These genetic stocks correspond well with the known distribution of larvae and provide support for the idea that striped marlin either exhibit spawning site fidelity and (or) are less migratory than other billfishes. Future studies of striped marlin should include more collection locations as well as more temporal replicates and surveys at spawning grounds, as they may reveal the presence of additional genetic stock structure. The presence of genetically independent stocks should be factored into management and conservation decisions impacting striped marlin.

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Accounting for biological and physical sources of acoustic backscatter improves estimates of zooplankton biomass

Joseph D. Warren and Peter H. Wiebe

Abstract: To convert measurements of backscattered acoustic energy to estimates of abundance and taxonomic information about the zooplankton community, all of the scattering processes in the water column need to be identified and their scattering contributions quantified. Zooplankton populations in the eastern edge of Wilkinson Basin in the Gulf of Maine in the Northwest Atlantic were surveyed in October 1997. Net tow samples at different depths, temperature and salinity profiles, and multiple frequency acoustic backscatter measurements from the upper 200 m of the water column were collected. Zooplankton samples were identified, enumerated, and measured. Temperature and salinity profiles were used to estimate the amount of turbulent microstructure in the water column. These data sets were used with theoretical acoustic scattering models to calculate the contributions of both biological and physical scatterers to the overall measured scattering level. The output of these predictions shows that the dominant source of acoustic backscatter varies with depth and acoustic frequency in this region. By quantifying the contributions from multiple scattering sources, acoustic backscatter becomes a better measure of net-collected zooplankton biomass.

Résumé : Afin de pouvoir convertir des mesures de rétrodiffusion d'énergie acoustique en estimations de l'abondance et en renseignements taxonomiques relatifs à la communauté zooplanctonique, il est nécessaire d'identifier tous les processus de rétrodiffusion en activité dans la colonne d'eau et de mesurer leurs contributions à la rétrodiffusion. Nous avons inventorié les populations de zooplancton sur les bords est du bassin de Wilkinson du golfe du Maine dans le nord-ouest de l'Atlantique en octobre 1997. Nous avons obtenu des échantillons au filet trainant à différentes profondeurs, des profils de température et de salinité, ainsi que des mesures de rétrodiffusion acoustique à des fréquences multiples dans les 200 m supérieurs de la colonne d'eau. Nous avons identifié, dénombré et mesuré les échantillons de zooplancton. Les profils de température et de salinité ont servi à estimer l'importance de la microstructure de la turbulence dans la colonne d'eau. Ces ensembles de données traités avec des modèles théoriques de diffusion acoustique ont permis de calculer les contributions respectives des agents biologiques et physiques de diffusion au niveau global de diffusion mesuré. Le résultat de ces prédictions montre que la source dominante de rétrodiffusion acoustique dans cette région varie en fonction de la profondeur et de la fréquence acoustique. Une fois calculées les contributions des sources multiples de diffusion, la rétrodiffusion acoustique permet de mieux mesurer la biomasse du zooplancton récolté au filet.

[Traduit par la Rédaction]

Introduction

Acoustic surveys of zooplankton and fish offer many advantages over other sampling techniques (Holliday and Pieper 1995; Foote and Stanton 2000). Measurements of acoustic backscatter using a scientific echosounder can be made from ships, buoys, or moorings and thus provide greater spatial coverage or longer time series than conventional sampling techniques such as net tows or diver observations. Although video sampling methods (Davis et al. 1996; Benfield et al. 2001, 2003) provide some of the same advantages as acoustics, they are not used as extensively as acoustic surveys and typically have much smaller sampling volumes.

One of the difficulties in using acoustic backscatter to measure marine life in the ocean is that the data collected are indirect measures of biota. If aggregations of animals in a region are monospecific and of similar size, theoretical backscatter models can be used to estimate their distribution and abundance (Hewitt and Demer 2000). If more than one frequency of sound is used, then more categories and size classes of animals may be distinguished and their abundance estimated (Martin et al. 1996; Brierley et al. 1998). However, these approaches require that the type of the scatterers

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present in the water column is known. Because of this, zooplankton samples are typically collected by net tows during an acoustic survey. These samples provide taxonomic and size information that can be used to predict the level of acoustic backscatter in the water column.

This prediction is often referred to as the forward problem (FP). For a particular type of backscatterer (usually delineated by either size or taxonomy), the scattering contribution can be found by multiplying the numerical density of scatterers (N_i, in m^{-3}) present and the backscatter cross section for an individual scatterer of this type (σ_i, in m^2). The backscatter cross section is a function of several parameters, including the size, shape, composition, and orientation of the scatterer and acoustic frequency. The output of the FP is the volume backscatter coefficient (s_v, in m^{-1}), which is found by summing the contributions from the different types of scatterers present

$$(1) \qquad s_v = \sum_i (N_i \sigma_i)$$

These calculated levels of backscatter are then compared with measured values from field surveys. Often, echosounders record the volume backscatter strength (S_v, in dB), which is related to s_v by

$$(2) \qquad S_v = 10\log_{10}(s_v)$$

If the measured and predicted values agree, the inverse problem (IP) (using measured scattering values and theoretical scattering models to determine the number, size, or type of animals present) is more likely to be solved correctly. However, the predicted and measured volume backscatter strengths occasionally differ by an order of magnitude (or more), which can lead to large errors or uncertainty in solving the IP. For example, a 3 dB difference in S_v corresponds to a factor of two difference in the number of animals or their biomass. In practice, solutions to the FP are used in a diagnostic sense to determine how well the theoretical scattering models predict observed levels of backscatter in the ocean (Wiebe et al. 1996, 1997). These results often indicate that only a small subset of the animals present in the water column are acoustically important or, in some cases, that backscatter predictions based on the sampled zooplankton are unable to account for all of the observed backscatter and that another scattering source is unaccounted for in the FP analysis (Mair et al. 2005).

Although nearly every acoustic survey relies on net tow data to ground-truth the acoustic data, many do not take complete advantage of all the available information provided by the net tow. Typically, FP calculations are performed to identify the acoustically dominant taxa in the water column; however, the quantification of backscatter contributions from all of the taxa found in the water column is rarely done. Although many taxa present in the water column will contribute negligibly to the overall level of backscatter (because of small size, low numerical density, or low scattering efficiency), there are often several scattering sources that contribute substantially to the overall level of measured acoustic backscatter. Furthermore, the vertical distribution of zooplankton in the water column varies, which may cause the taxa that is the largest acoustic scatterer to change as a function of depth. Many of the net tows used to ground-truth acoustic data cannot provide this information, which may further complicate estimates of zooplankton abundance or distribution.

Even if the backscatter from marine organisms is accurately measured, there are other processes in the ocean that can contribute measurable amounts of backscattered acoustic energy. Suspended sediments, air bubbles, ocean mixing processes, and other biota have all been observed during acoustic surveys of zooplankton (Wiebe et al. 1997; Trevorrow 1998); however, backscatter from nonbiological processes is rarely quantified during field surveys.

Acoustic methods have been used for many years to observe physical mixing processes in the ocean (Thorpe and Brubaker 1983; Orr et al. 2000; Ross and Lueck 2005). It has only been in the last decade that theoretical scattering models for these processes have begun to be tested in the field. These models use the variations in temperature and salinity to calculate changes in the index of refraction and density in the water column that result from turbulence and other mixing processes. The acoustic scattering that occurs from these variations can then be predicted (Goodman 1990; Seim et al. 1995; Lavery et al. 2003).

Depending on the mixing rates present (generally characterized by the dissipation rates of turbulent kinetic energy (ε) and temperature variance (χ)), backscatter from turbulent microstructure can be equal to or greater than that from assemblages of zooplankton, particularly at the lower range of frequencies commonly used for acoustic surveys (i.e., <100 kHz; see fig. 1 in Warren et al. 2003). Estimates of S_v from this mechanism range from –110 dB in calm waters with small temperature and salinity stratification to –60 dB or higher for regions of intense mixing such as the Bosporus Strait (Seim 1999).

This study examines the contributions of both biological and physical sources of backscatter to the water column in the Gulf of Maine. Contributions from each scattering source were quantified using theoretical scattering models and either net tow data or hydrographic profiles for multiple depth bins of the water column and multiple acoustic frequencies. The theoretical predictions of backscatter from the different scattering sources were used to correct the amount of measured backscatter in the water column to reflect scattering only from biological sources. The adjusted values of measured backscatter were then compared with measurements of zooplankton biomass.

Materials and methods

As part of a GLOBEC (GLOBal ocean ECosystem dynamics) process cruise studying the populations of *Calanus* in the basins of the Gulf of Maine, an acoustic survey was conducted in the eastern part of Wilkinson Basin (located between Georges Bank and Stellwagen Bank) in mid-October 1997 from the R/V *Endeavor* (Table 1). To provide spectral information about the acoustic backscatter processes occurring in the water column, multiple frequency acoustic backscatter data were collected by BIOMAPER-II (BIO-optical Multifrequency Acoustical and Physical Environmental Recorder) (Wiebe et al. 2002).

Multiple opening and closing net and environmental

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Table 1. Location and time of the CTD vertical profiles and MOCNESS tows used in this study collected in October 1997 from the R/V *Endeavor*.

Event	Julian year-day	Latitude (N)	Longitude (W)	Beginning or end
CTD 08	287.451	42°14.97′	68°44.77′	Beginning
	287.467	42°14.97′	68°44.77′	End
MOC 07	287.620	42°24.04′	68°49.03′	Beginning
	287.686	42°24.93′	68°44.22′	End
CTD 10	289.535	42°25.08′	68°44.49′	Beginning
	289.562	42°25.08′	68°44.49′	End
MOC 09	289.896	42°28.70′	68°45.00′	Beginning
	289.949	42°30.97′	68°46.69′	End

sensing system (MOCNESS) tows (Wiebe et al. 1985) were conducted to collect zooplankton samples while BIOMAPER-II was concurrently recording acoustic backscatter data from the water column. Profiles of the temperature and salinity of the water column were made with conductivity–temperature–depth (CTD) sensors onboard the MOCNESS and nearby higher-resolution CTD casts (Sea-Bird 911; Sea-Bird Electronics, Inc., Bellevue, Wash.) from the vessel. Data from two sampling periods are presented herein, with samples from year-day 287 (CTD 08, MOC 07) and year-day 289 (CTD 10, MOC 09). The CTD and MOCNESS sampling took place in the same general area (Table 1). These data sets (acoustic backscatter, zooplankton taxa and size, and temperature and salinity profiles) provided enough information to estimate the contributions from biological and physical sources of acoustic backscatter.

Acoustic backscatter measurements

BIOMAPER-II (Wiebe et al. 2002) is a towed body with numerous acoustic, environmental, video, and bio-optical sensors. The acoustic system consists of five pairs of transducers (operating at 43, 120, 200, 420, and 1000 kHz), with one of each frequency looking upward and the other downward. The transducers have depth ranges of 200, 200, 150, 100, and 35 m, respectively, with a vertical resolution of 1 m depth bins. Backscattered energy from each transducer and for each depth bin was recorded as echo-integrated volume backscattering strength every 12 s. At typical survey speeds, this ping rate corresponds to a horizontal range between pings of 20 to 50 m. The instrument was normally towed obliquely through the water column; however, because additional equipment was in the water during these measurements, the tow body was kept at a constant depth of approximately 5 m below the surface, and slower tow speeds resulted in a horizontal resolution between pings of approximately 10 to 15 m. Because of this configuration and the limited depth range of the 1 MHz transducer, only data from the downward-looking transducers at 43, 120, 200, and 420 kHz were analyzed.

The acoustic data were processed and combined with data from the ESS (environmental sensing system) sensors that are also onboard BIOMAPER-II. The acoustic system was calibrated using standard target spheres before the cruise. The final data file provided echo-integrated volume backscatter coefficients (s_v) for the water column along with date, time, position (latitude, longitude, instrument depth), temperature, salinity, fluorescence, turbidity, and other sensor data.

Zooplankton net sampling

Two 1 m^2 MOCNESS (Wiebe et al. 1985) tows were analyzed to identify, enumerate, and measure the zooplankton present in the waters of Wilkinson Basin. A MOCNESS system consists of a series of nine nets, which enables specific depth strata to be sampled. Generally, net 0 was open from the surface to the deepest point of the tow (10–20 m above the bottom), and the remaining nets (1–8) were opened and closed in succession every 25 to 50 m during the return to the surface. The MOCNESS system also recorded the volume of water filtered by each net, the time that each net was opened and closed, depth, salinity, temperature, density, and fluorescence.

The nets were equipped with 333 μm mesh and cod-end buckets for collection of zooplankton and larval fish. Each cod-end sample was split and stored in a buffered formalin solution. Postprocessing of the samples consisted of silhouette photography of the animals (Davis and Wiebe 1985). These images were then examined under a microscope and the organisms were measured and identified by taxonomic group. Numerical density and biomass ($mg \cdot m^{-3}$) were then calculated for each net for each taxonomic group (Davis and Wiebe 1985; Wiebe 1988).

Scattering models

Mathematical models that combine scattering physics and the geometry of the animal shape for several types of zooplankton were used to provide backscatter information for single animals for use in the FP analysis (Table 2). These models have been developed previously and only slight modifications to some input parameters have been made in this work. These modifications were limited to body length–width relationships and the use of a simple fluid-like tissue model for certain gelatinous animals. The models represent the three main taxonomic types of zooplankton: fluid-like, elastic-shelled, and gas-bearing animals (Stanton et al. 1998).

Biological scatterers

Fluid-like models were used for copepods, euphausiids, amphipods, and other animals that have a thin shell (which does not support a shear wave) and a body composition that has similar density and sound speed to that of sea water. Fluid-like animals, which constituted the majority of zooplankton taxa that were encountered in the Gulf of Maine and Georges Bank regions, were modeled as weakly scattering, bent, fluid cylinders (Stanton et al. 1993*b*; Chu et al. 1993). The model has input parameters of animal size (typically, a, the radius), the acoustic frequency (f) or wave number ($k = \frac{2\pi f}{c}$, where c is the speed of sound in seawater), the ratio of sound speed and density between the scatterer and the surrounding fluid (g and h, respectively), the length–width ratio of the animal (L_0), and the orientation of the cylinder relative to the acoustic wave front. An assumed range of orientation angles based on previous studies was used in modeling the euphausiids, as acoustic backscatter strength is a function of animal orientation (Sameoto 1980; McGehee et al. 1998; Warren et al. 2002). These animals

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Table 2. Citations and parameters for the acoustic backscattering models used in the forward problem calculations.

Scatterer	Scattering model citations	Scattering model parameters
Euphausiids	Stanton et al. 1993*a*, Stanton and Chu 2000	β_D = 5.3576, R = 0.058, "head-up" orientation distribution (20° ± 20°)
Copepods and larval crustaceans	Stanton et al. 1993*a*, Stanton and Chu 2000	β_D = 2.5497, R = 0.058, uniform orientation distribution (0°–360°)
Amphipods	Stanton et al. 1993*a*, Stanton and Chu 2000	β_D = 3.0021, R = 0.058, uniform orientation distribution (0°–360°)
Polychaetes and chaetognaths	Stanton et al. 1993*a*, Stanton and Chu 2000	β_D = 17.151, R = 0.058, uniform orientation distribution (0°–360°)
Limacina pteropods	Stanton et al. 1994	R = 0.5
Siphonophore nectophores	Monger et al. 1998	R = 0.028
Siphonophore pneumatophores	Anderson 1950	
Salps	Monger et al. 1998	R = 0.028
Microstructure	Seim 1999	

Note: β_D is the length–width ratio (L/D) of the animal, R is the reflection coefficient.

often orient in a slight head-upward posture and were modeled with a 20° ± 20° orientation distribution, where 0° is broadside orientation.

Modeling of other fluid-like animals was similar to that for the euphausiids. The value of β_D was changed slightly to better reflect the body shapes of the other fluid-like animals (Table 2). Although Benfield et al. (2001) indicated that copepods may tend to orient themselves vertically in the water column, it was not known under what conditions this occurs, so an average over all orientations (uniform distribution) was used for all fluid-like animals except for euphausiids. Small changes in the values of g and h can cause large variations in the level of scattering from an animal (Chu et al. 2000; Chu and Wiebe 2005), so to minimize variability in this analysis, constant values of g = 1.0357 and h = 1.0279 (Foote 1990) were used for all fluid-like animals. It is not known whether the fluid-like animals found in this region have similar material properties as few data are available for animals other than copepods and euphausiids. If differences in the material properties exist for the fluid-like animals, this would cause larger variations in the predicted level of biologically caused backscatter.

Elastic-shelled models were used for animals with hard, elastic shells such as pteropods. Pelagic pteropods are typically very small (<1 mm in diameter) but scatter a large amount of sound (per unit biomass) because of their dense shell. Other strong scatterers are gas-bearing animals such as siphonophores, where the scattering is caused by small gas bubbles used for buoyancy. Gelatinous animals (e.g., salps or medusae) or parts of animals (e.g., siphonophore nectophores) have not been modeled as thoroughly as the fluid-like or elastic-shelled animals. However, because of their body composition, it is believed that they scatter smaller amounts of sound by a mechanism similar to that of the fluid-like animals (Monger et al. 1998).

Several of these zooplankton models have been compared with measured scattering from individual animals (copepods, euphausiids, pteropods, and siphonophore nectophores and pneumatophores) (Stanton and Chu 2000; Stanton et al. 2000; Warren et al. 2001). The remaining zooplankton models have not been tested specifically against measurements from individual animals (amphipods, salps, polychaetes, chaetognaths, larval crustaceans, and cyphanautes); however, these groups contain animals that are believed to be less important acoustically in this study because of either low numerical densities or very weak scattering characteristics.

The zooplankton backscatter models were combined with the abundance data from the MOCNESS tows to estimate the amount of biologically caused scattering in the water column. For each animal type collected in a net tow, the backscatter contribution for an individual animal was determined using the appropriate scattering model. These contributions were summed over all animals collected and then divided by the volume of water sampled by the net to arrive at a volume backscatter coefficient for the depth stratum of the net. The contributions from all of the zooplankton were summed and the result was a predicted volume backscatter coefficient for biological sources.

Physical scattering processes

Additional sources of backscatter that have been observed in the vicinity of Georges Bank include suspended sediments and bubbles. These scatterers are not believed to be important in this study because of the absence of sediment in net tows and the relatively calm sea state during the survey period. However, internal waves were seen in the acoustic record during the survey and thus the importance of backscatter from the resultant turbulent microstructure was examined.

Scattering from turbulent microstructure in the water column was analyzed in a parallel manner to that from zooplankton except that hydrographic data are used as the scattering model input instead of net tow data. Predictions from the theoretical backscatter model were made based on inputs of temperature, salinity, and the dissipation rates of turbulent kinetic energy and temperature variance (Seim 1999). The latter two values were estimated using temperature and salinity profiles from CTD casts taken either before or after the MOCNESS tow (Table 1) (Thorpe and Brubaker 1983; Warren et al. 2003). Although the ESS system on the MOCNESS provided temperature and salinity profiles, the

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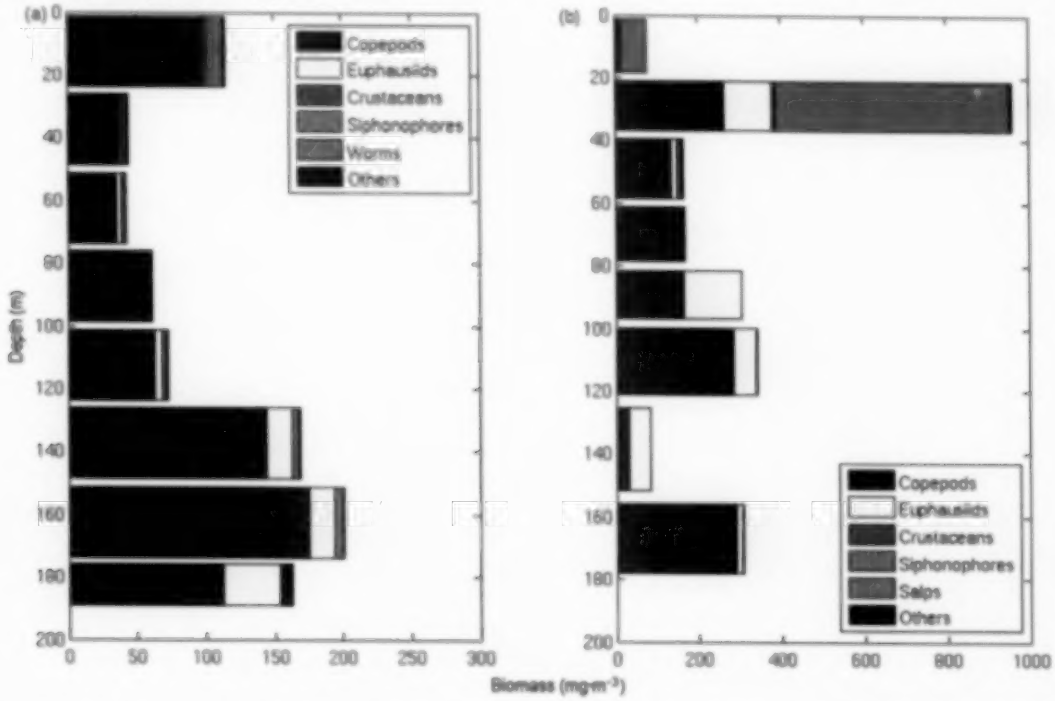
Fig. 1. Total biomass estimated from (*a*) MOCNESS 7 and (*b*) MOCNESS 9 data with the relative contributions of the different taxonomic groups. Several taxa are grouped together in the plot, including amphipods and other crustaceans, including larvae ("Crustaceans"), polychaetes and chaetognaths ("Worms"), and pteropods and gelatinous zooplankton ("Others"). The vertical thickness of the bar corresponds to the depth range sampled. For MOCNESS 7, the lower depths were composed primarily of copepods and euphausiids with some gelatinous animals, whereas the surface layers also contained small amounts of polychaetes, chaetognaths, and siphonophore fragments. MOCNESS 9 sampled a large subsurface layer of salps (over 2500 animals·m^{-3}), which dominated the biomass sample. The remaining nets were composed primarily of copepods and euphausiids.



sampling rate was limited to 0.25 Hz, thus to resolve temperature and salinity variations at vertical scales less than 1 m, the higher resolution CTD cast data (sampled at 24 Hz) were used.

The CTD data were not collected concurrently with the acoustic and net tow data so there are potential errors in using the CTD data to describe the structure of the water column when the net and acoustic data were recorded. However, hydrographic profiles for each CTD cast were consistent with the profile recorded by the corresponding MOCNESS tow. Although this method is far from ideal for measuring values of turbulent kinetic energy and temperature variance, this method has been used previously to make realistic estimates that compare favorably to measurements of turbulent kinetic energy and temperature variance made in a similar region (Warren et al. 2003; Seim 1999). Sea state was relatively calm during these tows so we believe that errors due to vertical ship and CTD sensor movement are minimal. The estimated level of backscatter from microstructure was then averaged over the depth ranges sampled by each MOCNESS net so that it could be compared with the FP estimates from the zooplankton.

Results

Data are presented for two MOCNESS tows that occurred in nearby regions but differed in the types of zooplankton present, levels of acoustic backscatter, and water column structure. MOCNESS 7 was lowered to a depth of 191 m and was brought to the surface with a net closed and new net opened at 175, 150, 125, 101, 74, 50, 26, and 0 m. The lower nets contained large amounts of biomass (150–200 mg·m^{-3}) and were dominated by copepods and euphausiids (Fig. 1*a*). The surface layer (0–26 m) had a higher level of biomass (over 100 mg·m^{-3}) than the other upper water column samples and was composed of copepods, polychaetes, chaetognaths, and amphipods.

Data from MOCNESS 9 show a different depth distribution of biomass, as well as a slightly more diverse taxonomic composition (Fig. 1*b*). Nets were opened and closed at depths of 180, 153, 124, 99, 80, 60, 39, 20, and 0 m. The zooplankton collected were dominated by an enormous number of salps (2500 animals·m^{-3}) near the surface (from depths of 20–40 m), resulting in a large amount of biomass, nearly 1 g·m^{-3}. There was also a substantial amount of biomass from 80–124 m that was composed of copepods and euphausiids, as well as a copepod-dominated bottom layer. The salp surface layer was an unusual occurrence on this cruise, and no other net sample from the nine MOCNESS tows collected during the cruise had such a large amount of biomass. For both net tows, the dominant component of biomass at most depths were calanoid copepods.

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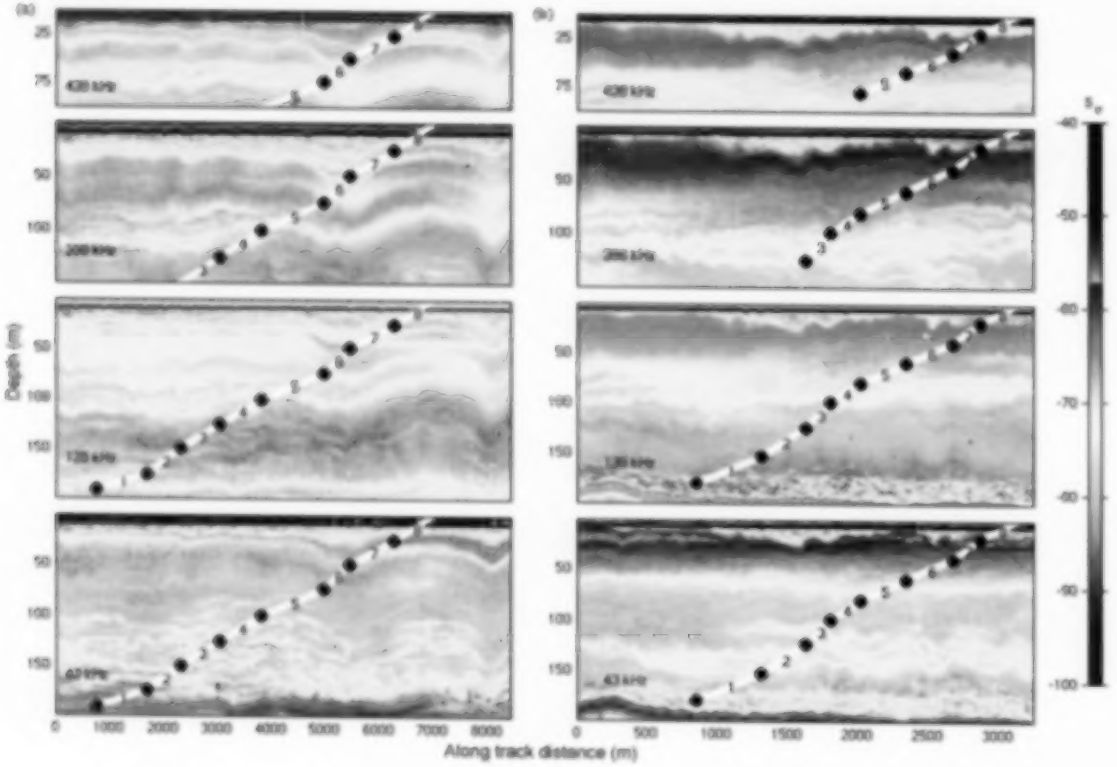
Fig. 2. BIOMAPER-II echograms for 43, 120, 200, and 420 kHz for (*a*) MOCNESS 7 and (*b*) MOCNESS 9. The white line indicates the path of the net system; black circles indicate where nets were opened and closed. Different regions of the water column have different measured backscattering strengths for the various frequencies. Remnants of an internal wave (undulating backscattering layers) were observed during MOCNESS 7, whereas MOCNESS 9 measured a strong near-surface layer at a depth of 20 m, which is seen most strongly in the 43 kHz echogram.



As each MOCNESS tow was being conducted, BIOMAPER-II collected acoustic data while being towed at a depth of approximately 5 m beside the ship. The acoustic data were offset horizontally from the MOCNESS samples by the amount of wire out on the net tow (at most a few hundred metres). To compare the acoustic regions with the MOCNESS information, the trajectory of the MOCNESS was overlaid on the acoustic plot to determine where each net sampled (Fig. 2).

The acoustic data for MOCNESS 7 shows strong backscatter at the higher frequencies and weaker backscatter at 43 kHz for much of the water column, although this pattern is reversed for the surface and deepest waters sampled (Fig. 2*a*). Remnants of an internal wave were observed in the echogram, and the upper layer of the wave, sampled by nets 7 and 8, had the strongest backscatter at the lowest acoustic frequency, whereas backscatter from the thick layer between 50 and 100 m was strongest at the highest frequencies. The echogram collected for MOCNESS 9 showed a midwater scattering layer that was sampled by nets 4–6, with backscatter that had a similar relationship between scattering strength and acoustic frequency (Fig. 2*b*). This frequency dependence is consistent with the backscatter model used for fluid-like scatterers (Warren et al. 2003). A near-surface scattering layer (sampled by net 7) shows the opposite effect (strongest backscatter at lower frequencies), which indicates that the scattering was dominated by a different type of scatterer, possibly the large amount of salps or physical processes occurring at the thermocline.

The water column profile for MOCNESS 7 showed a well-mixed region from 20–60 m with a steep temperature and salinity gradient above this layer and a shallower gradient below (Fig. 3*a*). These mixing processes likely contributed to the backscatter observed between 15 and 100 m in the echogram (Fig. 2*a*). The hydrographic data for MOCNESS 9 showed a well-mixed region in the upper 20 m of the water column, with a large gradient in temperature, salinity, and density occurring in the next 10 m (Fig. 3*b*). There were several regions of potential or recent mixing (shown by unstable or nearly vertical sections of the density profile) occurring between 0–20 m, 60–100 m, and 140–180 m, although there were smaller instabilities that occurred throughout the profile.

When FP predictions of backscatter were examined for the individual contributions for different animals or processes, the dominant scatterers for MOCNESS 7 were turbulent microstructure, euphausiids, and siphonophore pneumatophores; however, the amphipod category (which included other larval crustaceans) and chaetognaths also caused appreciable amounts of backscatter (Fig. 4). The

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Fig. 3. Temperature (solid line), salinity (broken line), and density (dotted line) profiles collected by the ESS system onboard (*a*) MOCNESS 7 and (*b*) MOCNESS 9. Regions of potential mixing and turbulent microstructure are indicated by nearly vertical or unstable sections of the density profile, e.g., 30–80 m for MOCNESS 7 and 0–20 m, 60–100 m, and 140–180 m for MOCNESS 9.

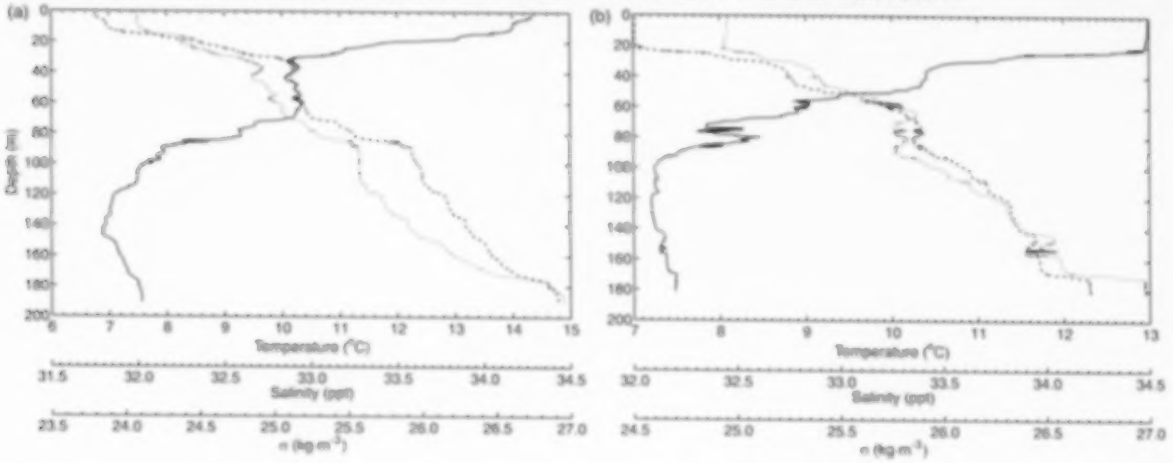

Fig. 4. Forward problem (FP) calculations for each class of scatterer for MOCNESS 7. The different acoustic frequencies (43, 120, 200, and 420 kHz) are represented by squares, stars, circles, and diamonds, respectively. Small copepods had body lengths less than 2.5 mm. Data points that lie above the diagonal line indicate that the FP underestimates the scattering, whereas points below the line are overestimates. Microstructure, siphonophore pneumatophores, chaetognaths, and euphausiids are the strongest contributors to the predicted levels of backscattering.

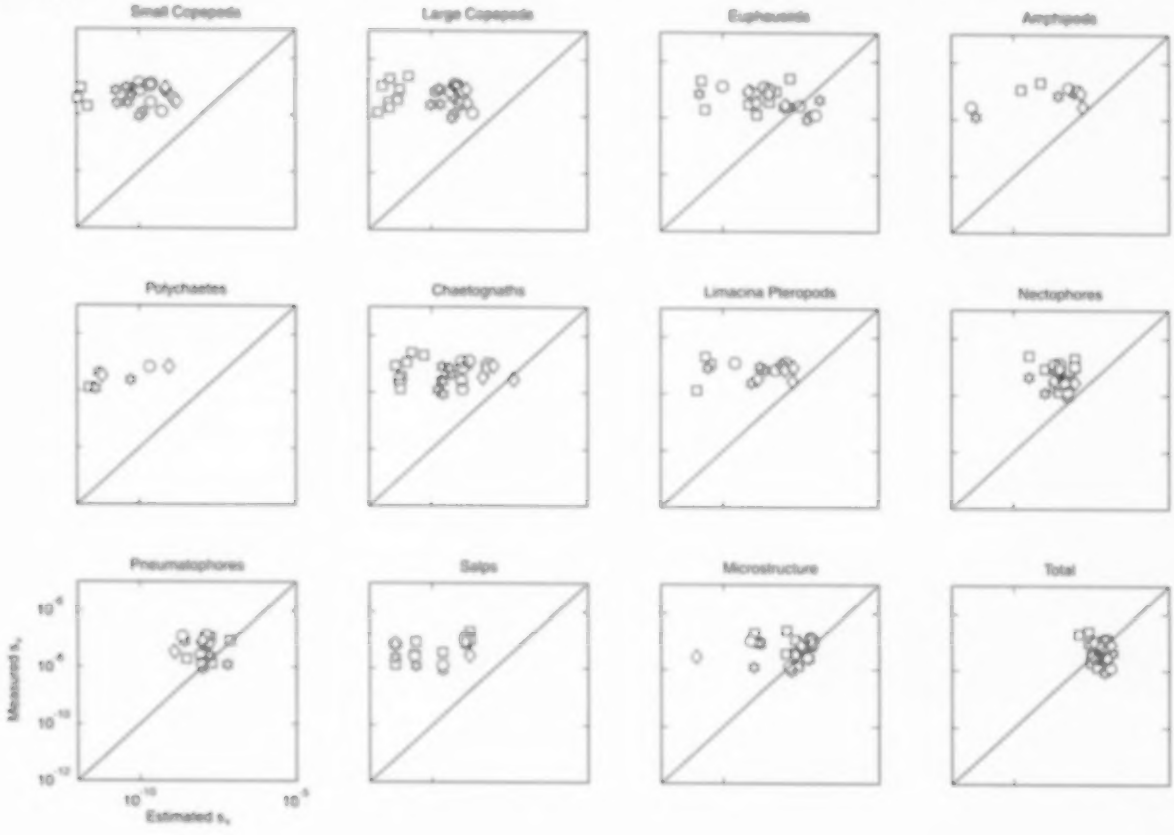

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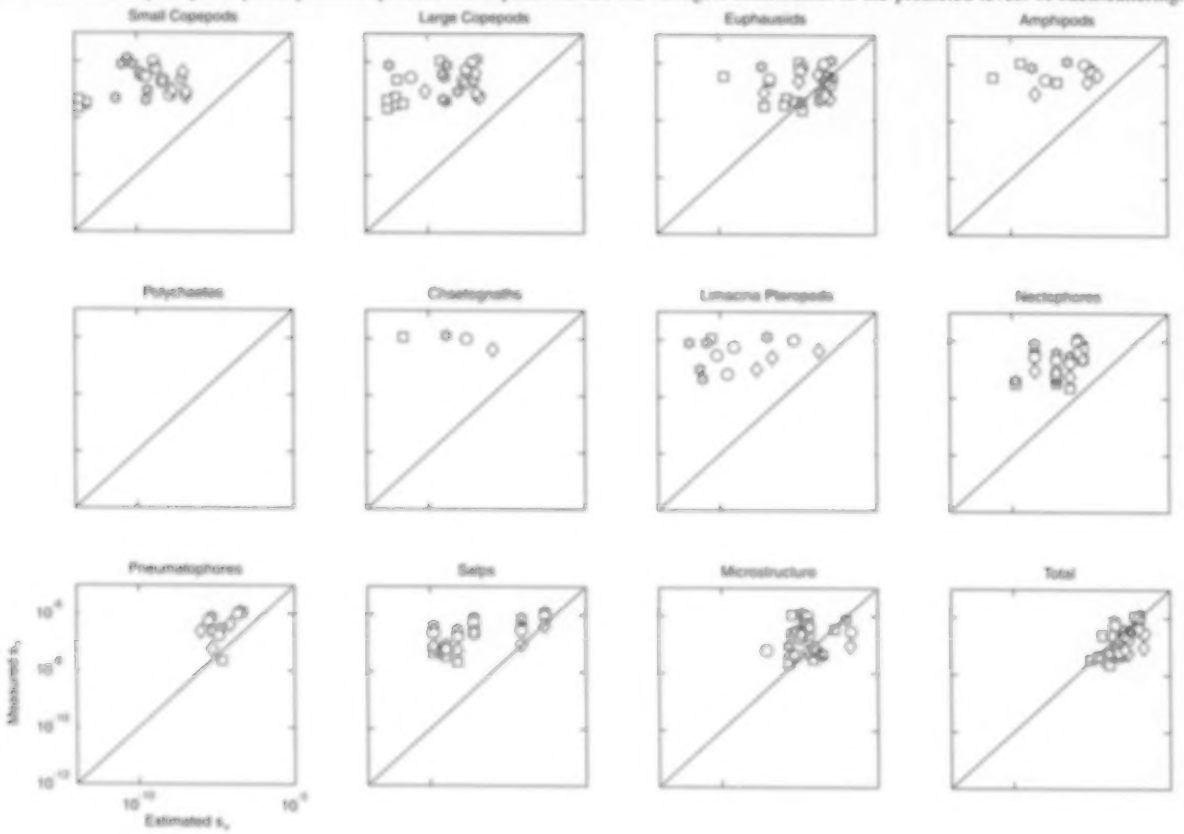
Fig. 5. Forward problem (FP) calculations for each class of scatterer for MOCNESS 9. The different acoustic frequencies (43, 120, 200, and 420 kHz) are represented by squares, stars, circles, and diamonds, respectively. Small copepods had body lengths less than 2.5 mm. Data points that lie above the diagonal line indicate that the FP underestimates the scattering, whereas points below the line are overestimates. Microstructure, salps, siphonophore pneumatophores, and euphausiids are the strongest contributors to the predicted levels of backscattering.



other animals (particularly the abundant copepods) contributed little to the overall predicted scattering except at the highest frequencies. Copepods also contributed little to the predictions for MOCNESS 9. Turbulence, euphausiids, salps, and siphonophore pneumatophores were the largest contributors to the backscatter (Fig. 5). It is striking that the copepods that were by far the largest contributors to biomass have such a small contribution to the predicted levels of backscatter. This is primarily a function of copepod size (a few millimetres in length) and acoustic frequency or wavelength. For the frequencies used in this survey, copepod backscatter is primarily a function of animal size, and despite their numerical abundance in the net tow data, they are simply too small to contribute much to the predicted level of backscatter except at the higher frequencies.

The MOCNESS data provided information about the contributions that different zooplankton taxa make to the overall amount of biomass. Similarly, the relative contributions of different biological and physical sources to the total amount of predicted backscatter in the water column can be made by combining MOCNESS data, CTD data, and backscatter models. The relative contribution of each scattering source (each animal taxa and microstructure) was calculated for each MOCNESS net depth range and BIOMAPER-II frequency (Figs. 6 and 7). The percentage contribution to the total predicted backscatter strength was found by dividing the predicted volume backscatter coefficient for each scatterer type by the overall calculated backscatter prediction. The percentage of total predicted scattering from physical (nonbiological) sources was calculated. The measured level of scattering (from the BIOMAPER-II data) was then reduced by this percentage to arrive at a corrected amount of measured backscatter that is believed to be from biological scatterers.

For example, the measured level of backscatter for MOCNESS 9 from depths of 0–20 m at 120 kHz is s_v = 1.58×10^{-6} m^{-1} (S_v = –58.0 dB). From Fig. 7, only 8% of the predicted backscatter for this sample is from biological sources. By multiplying the percentage of biologically caused predicted backscatter and the measured level of backscatter, an estimate of the biologically caused scatter in the water column was made, s_v = 1.26×10^{-7} m^{-1} (S_v = –69.0 dB).

Nonbiological backscatter contributions were important for several regions sampled by MOCNESS 7 (Fig. 6). The predicted contributions from microstructure were largest for

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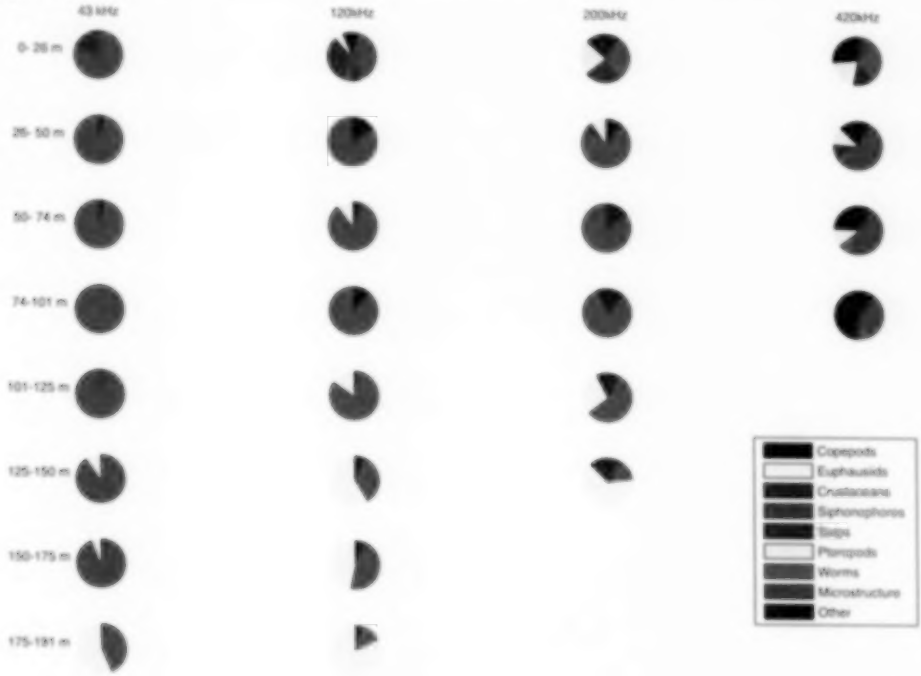
Fig. 6. Percentage breakdown of forward problem calculations for each net and acoustic frequency of MOCNESS 7. Turbulent microstructure contributes large amounts to the total backscattering in the midwater regions, whereas euphausiids backscatter a majority of the sound in the deeper water. Siphonophores contribute greatly at the lower frequencies, but not at the higher ones. The surface region (0–20 m) has a very diverse group of scatterers.



the region between 20 and 100 m (which again corresponded to regions of mixing indicated in the hydrographic data), whereas euphausiids were the main scatterers for the deepest nets. Siphonophores contributed to the backscatter more for lower acoustic frequencies and were negligible at the highest frequency. The backscatter in the near surface was the most diverse with regard to scatterer type, with nearly all taxonomic types contributing.

For MOCNESS 9, the deeper water column and surface layer were dominated by scattering from microstructure (Fig. 7). These large backscatter contributions from turbulent microstructure occurred in the same regions that the hydrographic profile data indicated was well mixed (0–20 m and 140–170 m). Euphausiids were the dominant scatterers in the midwater depths, with siphonophore pneumatophores and nectophores also contributing. Salps were extremely weak scatterers, and although outnumbering the other animals and dominating the biomass in the near-surface region, they contributed only 30%–60% to the total backscatter in that region.

If the FP is well posed and one taxa dominates the measured scattering, then there should be a relationship between biomass and measurements of backscatter strength. Both biomass and volume backscatter cross sections (s_v) are linear functions (for a particular taxa) of the number of animals. Therefore it is likely that a relationship between biomass and volume scattering strength should exist. The relationship between biomass and measured backscatter may not be linear, however, if more than one scattering process (or taxonomic type or size class) is substantially contributing to the measured backscatter.

The biomass and acoustic backscatter data sets from both MOCNESS tows were combined, and the regression between the logarithm of biomass and measured acoustic backscatter strength (S_v, a logarithmic measure of acoustic backscatter) was found for each acoustic frequency. The log of both biomass and backscatter was used as some of the acoustic data (specifically predicted backscatter for some scatterer types) ranged over nearly five orders of magnitude. The backscatter model used for salps has not been as well tested, by comparing theoretical backscatter predictions with measured backscatter from individual animals, as the backscatter models used for other animals. Because of this fact and the extremely high biomass of salps caught in net 7 of MOCNESS 9, the data from this net were not included in this analysis.

There was not a strong relationship (all r^2 values < 0.4) between log-transformed biomass and measured backscatter levels for any of the four frequencies used (Fig. 8). A poor relationship between zooplankton biomass and acoustic backscatter is likely to occur when nonzooplankton scatterers are contributing to the measured amount of acoustic backscatter. This result was not surprising as turbulent microstructure was predicted to contribute greatly to the measured backscatter for some portions of the water column.

A similar analysis was performed for biomass and biologically caused backscatter (Fig. 9). When backscatter attributed to physical processes was removed, the relation-

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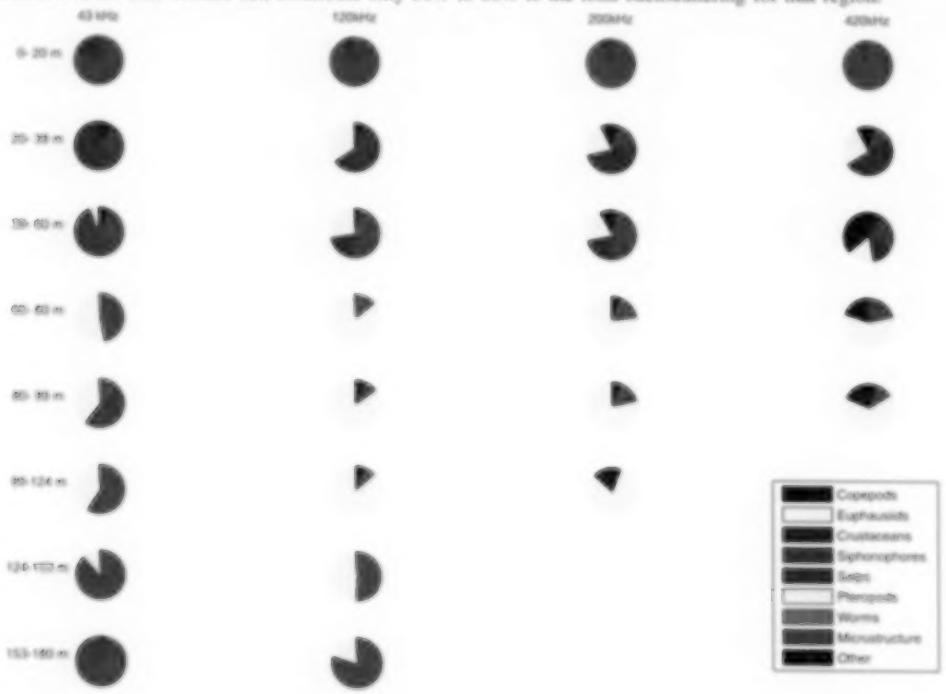
Fig. 7. Percentage breakdown of forward problem calculations for each net and acoustic frequency of MOCNESS 9. Euphausiids dominate the backscattering in the midwater depths, whereas turbulence contributes strongly near both the bottom and the surface. The salps, which dominated the biomass in the near-surface net, contribute only 30% to 60% to the total backscattering for that region.



ship between log measures of biomass and acoustic backscatter was more linear. Regression coefficients improved for all frequencies, indicating a better correlation between biomass and backscatter. It must be noted that the regression coefficients for each frequency are still fairly small (r^2 ranged from 0.38 to 0.52); however, these values are a factor of two or three larger than if the source of the backscatter is not identified. By accounting for the source of acoustic backscatter using hydrographic and net tow information, this method can be used to improve the use of acoustic backscatter data as a measure of zooplankton biomass.

Discussion

One of the goals of acoustic surveys is to estimate zooplankton biomass, and this requires that the relationship between biomass and acoustic backscatter is well understood. The data presented here indicate that improvements can be made in the interpretation of field-collected survey data if the contributions of all scattering sources are quantified. The relative importance of physical and biological sources of acoustic backscatter will vary with location in the ocean, and certainly, some regions will not have substantial backscatter from physical processes in the water column, whereas other areas (such as the sites in this study that have internal waves present) will have significant contributions to the backscatter from nonbiological sources. The modeling efforts outlined in this work provide one way of determining if physical sources of backscatter will need to be accounted for when interpreting acoustic backscatter survey data. However, these improvements are just one step of many that need to be taken in order that acoustic surveys may provide estimates of zooplankton abundance that are accurate and ecologically useful. Given that biomass and predictions of biologically caused backscatter are not perfectly correlated, sources of error in the analysis, such as inaccuracies in the backscatter models used, must be examined.

The zooplankton backscatter models for fluid-like, elastic-shelled, and gas-bearing zooplankton have been used previously in the analysis of field-collected data (Wiebe et al. 1996, 1997; Greene et al. 1998); however, there are many variables used in these models that are inadequately understood, such as animal behavior and orientation or the material properties (g and h) of the zooplankton. A better understanding of the scattering model inputs would reduce errors associated with these types of animals. Furthermore, there are numerous types of animals (salps, polychaetes, chaetognaths, gelatinous zooplankton) with backscatter characteristics that have been neither modeled nor measured in a laboratory environment.

Uncertainty about the inputs to the backscatter models is a concern for the microstructure model as well. Proper instrumentation was not present to measure the dissipation rates of turbulent kinetic energy and temperature variance, which are vital inputs into the theoretical microstructure of backscatter models, so the method used to estimate these inputs was not ideal. Although this method provided reasonable estimates of ϵ and χ, it likely overestimated the scattering contributions from microstructure. For example, some

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Fig. 8. The relationship between the logarithm of biomass and measurements of acoustic backscatter strength for MOCNESS 7 (squares) and MOCNESS 9 (circles), excluding net 7 from MOCNESS 9. Most frequencies show little correlation between these two variables, except for the highest frequency (420 kHz).

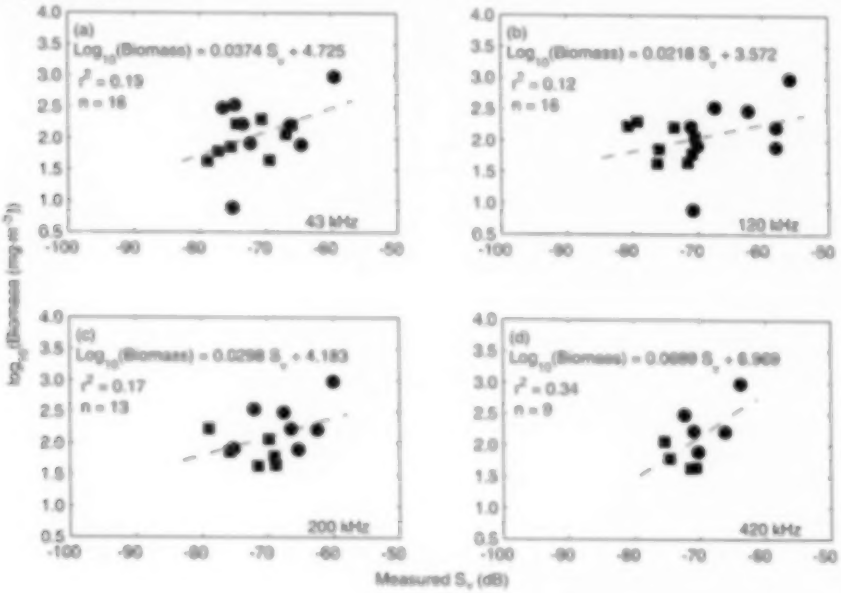
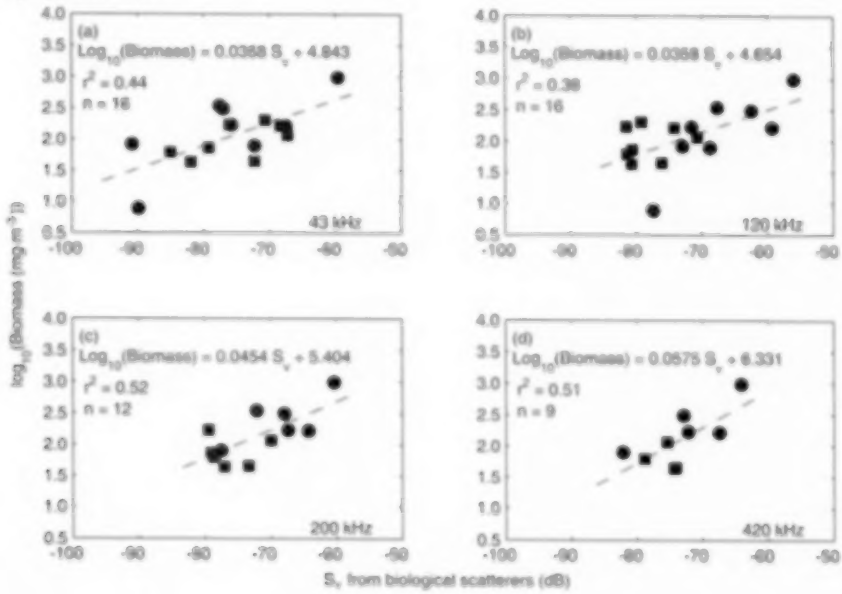


Fig. 9. The relationship between the logarithm of biomass and predictions of biologically caused acoustic backscatter for MOCNESS 7 (squares) and MOCNESS 9 (circles), excluding net 7 from MOCNESS 9. All of the acoustic frequencies show improved regressions and fairly linear relationships for the data.



regions of MOCNESS 9 have microstructure-caused s_v values that were larger than the backscatter measured by BIOMAPER-II. Further complicating this issue is the possibility that the vertical migration of animals may be creating significant amounts of turbulence and mixing (Huntley and Zhou 2004; Kunze et al. 2006).

Other possible sources of error in the FP analysis include erroneous zooplankton abundance and composition data and inaccurate measurements of the acoustic backscatter. Net tow information from MOCNESS systems has been used for several decades, and sampling errors from it are likely limited to net avoidance by large zooplankton (Wiebe et al.

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2004) and gelatinous animals being destroyed by the net mesh. Finally, the undersampling of animals by either nets (e.g., large euphausiids or small fish) or lower acoustic frequencies (e.g., copepods) will cause errors in the FP analysis. One approach that has been used in the analysis of acoustic scattering data (Warren et al. 2003) is to use the net tow estimates of numerical density as a lower bound on the true value (as you cannot have more animals in a net than are present in the water column) and to use the acoustically inferred estimates of biomass as an upper bound (as these rely on measures of backscatter strength that likely contain contributions from other scattering sources). In this manner, combining acoustic and net tow data can provide an upper and lower estimate of the abundance of zooplankton in the water column.

This study also demonstrates the importance of resolving the changes in biological and physical backscatter sources within the water column. A multiple net system, or other methods such as video or optical ground-truthing, may be necessary to accurately assess acoustic surveys of zooplankton biomass, particularly where the taxonomic components of the zooplankton community are diverse. Providing this vertical resolution and ground-truthing of the acoustic data also allows us to observe partitioning of the water column into different habitats that would not be apparent from either the acoustic data alone or a vertically integrating net tow. Although some regions of the ocean do have patches with a single dominant taxa (e.g., Antarctic krill), the variation in abundance and distribution of zooplankton taxa observed over a 200 m vertical span in this study demonstrates the importance of measuring and quantifying these changes.

The difference between predictions of S_v and those values that would perfectly correlate with the biomass data are on the order of 5–10 dB (assuming that the biomass data are accurate). These differences become very large when backscatter strengths are converted to estimates of biomass; therefore, these predictions result in estimates of zooplankton biomass that are correct to roughly an order of magnitude. In certain cases, this level of error may be acceptable, but further work is needed to reduce this uncertainty. Without accounting for the source of acoustic scattering in the water column, estimates of biomass from acoustics are likely to have even larger errors.

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Incorporating environmental variability in stock assessment: predicting recruitment, spawner biomass, and landings of sprat (*Sprattus sprattus*) in the Baltic Sea

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Abstract: Temperature has a significant positive impact on recruitment of sprat, *Sprattus sprattus*, in the Baltic Sea. Here we evaluate whether an existing recruitment model for the year classes 1973–1999 can forecast recruitment for five new year classes. The coefficient of variation (CV) of predictions was 5%, and four of five new year classes were within 95% confidence limits of predictions made by the earlier model. We then assimilated climatic, oceanographic, and recruitment linkages and their uncertainty into the standard International Council for the Exploration of the Sea (ICES) assessment procedure to predict key advisory-related variables such as spawning stock biomass (SSB) and landings. These linkages enable a forecast of recruitment earlier than the annual assessment meeting. Forecasts made using the North Atlantic Oscillation to predict the 2006 year class showed that spawner biomass would be 15% lower than spawner biomass calculated using the ICES standard methodology. The difference in perception of future biomass does not affect the advice for the stock because the spawning stock biomass is greater than the critical biomass limit (SSB > B_{PA}). However, when this is not the case or when it is desirable to broaden the ecosystem basis for fisheries management, incorporation of knowledge of recruitment processes may be beneficial.

Résumé : La température a un effet positif significatif sur le recrutement du sprat, *Sprattus sprattus*, dans la Baltique. Nous vérifions ici si un modèle préexistant du recrutement des classes d'âge de 1973–1999 peut prédire le recrutement pour cinq nouvelles classes d'âge subséquentes. Le coefficient de variation (CV) des prédictions est de 5 % et quatre des cinq nouvelles classes d'âge se situent dans l'intervalle de confiance de 95 % des prédictions du modèle antérieur. Nous incorporons ensuite les liens climat–océan–recrutement et leur incertitude dans la procédure courante d'évaluation du Conseil international pour l'exploration de la mer (CIEM, « ICES ») pour prédire les principales variables, telles que la biomasse du stock reproducteur (SSB) et les débarquements, qui servent à émettre des avis. Ces liens permettent de faire une prédiction du recrutement avant la tenue de la réunion annuelle d'évaluation. Les prédictions faites à l'aide de l'oscillation nord-atlantique concernant la classe d'âge de 2006 ont montré que la biomasse des reproducteurs serait de 15 % inférieure à la biomasse des reproducteurs calculée par la méthodologie standard de l'ICES. La différence de prévision de la biomasse future n'affecte pas les avis émis concernant le stock, car SSB > B_{PA}. Cependant, lorsque ce n'est pas le cas, ou lorsqu'il est souhaitable d'élargir la base écosystémique pour des fins d'aménagement de la pêche, l'incorporation de la connaissance des processus de recrutement peut être bénéfique.

[Traduit par la Rédaction]

Introduction

A large number of field and statistical studies have demonstrated that variations in ecosystem components affect recruitment in populations of fish and shellfish. These processes include effects of climatic, oceanographic, and biotic variables on the growth and survival of early life history stages (Heath 1992; Leggett and deBlois 1994; Bakun 1996). However, there still remain few examples in the literature where such knowledge is operationally used to make predictions of key variables of fisheries assessment and management (e.g., recruitment, spawner biomass, and landings by commercial fishing fleets (Myers 1998; Brander 2003; Ulltang 2003). Most of these examples include species with relatively short life spans, e.g., squid, sardine, or prawns, the management of which depends critically on the strength of incoming year classes or a small number of year classes (Agnew et al. 2002; International Council for the Exploration of the Sea (ICES) 2007), although examples with longer-lived species also exist (Bailey and Spring 1992; Megrey et al. 2005).

Here we explore how and whether environmental information could be used in the short-term management of a

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fish population. The species and ecosystem that we use as a case study is sprat (*Sprattus sprattus*) in the Baltic Sea. Sprat has a moderately long life span (ca. 8–10 years; maturity at ages 1–2 years) and is intensely exploited (mean landings were 362 000 tonnes (t) per year in 2003–2005). One-year-old sprat (recruits) contributed, on average, 26% in numbers and 15% in weight to the commercial landings in 2003–2005 (ICES 2006). Spawner biomass is presently above safe biological limits (ICES 2006), but there is no significant relationship between spawner biomass and recruitment (ICES 2006). As a consequence, recruitment forecasts are presently made using the geometric mean over a long time period (ICES 2006). Field and laboratory studies have been intensified in the past 10 years to identify how spawner biomass, egg production, and environmental variables influence recruitment. Results from these studies could help parameterize new stock–environment–recruitment models in the future.

These studies have shown that water temperature and several processes affected by temperature influence sprat reproduction (e.g., development of gonads in mature sprat, sexual and gonadal maturation; Grauman and Yula 1989; Kraus et al. 2003; Nissling 2004) and survival of eggs, larvae, and age-0 groups (Ojaveer 1998; Köster et al. 2003; Baumann et al. 2006). Statistical comparisons of recruitment with temperature and information gathered from these studies show that recruitment is significantly positively correlated with temperature (MacKenzie and Köster 2004). This relationship, derived for the years 1973–1999, was evident using an independent time series of catch-derived recruitment indices (1955–1972); in total, the temperature–recruitment relationship to date has been consistent (i.e., significant and positive) for 45 years. A retrospective analysis showed that recruitment predicted by environmental variables (i.e., water temperature, a winter index of the North Atlantic Oscillation (NAO), and Baltic Sea ice coverage) yielded smaller and less variable deviations from recruitment estimated with extended survivor analysis in later assessments than recruitment estimated with methodology currently used by ICES and that these environmental variables themselves are significantly intercorrelated (MacKenzie and Köster 2004).

In this study, we investigate whether these relationships are still valid for year classes born after 1999. We then investigate how the standard short-term predictions used in ICES advice would be affected by incorporating this ecosystem information into its methodology. As we demonstrate below, including such information requires careful consideration of when the environmental information can be provided relative to the annual stock assessment schedule for the stock in question (see also Planque et al. 2003). We have overcome this difficulty by using the seasonal chain of links between climate variability, sea conditions, and sprat recruitment (MacKenzie and Köster 2004).

Materials and methods

We first provide a description of the standard temporal schedule of assessment and prediction as conducted annually by ICES for this stock. We then conduct a series of statistical analyses. These analyses update existing relationships between recruitment and environmental variables and evaluate the sensitivity of short-term predictions of recruitment, spawner biomass, and landings to assumptions about environmental variability. Further details are included in relevant sections below.

ICES annual assessment cycle

The analyses below extend our earlier work, which demonstrated that recruitment of sprat in the Baltic Sea is positively influenced by temperature. However, because our original objective in that study was not only to identify ecosystem variables that might influence recruitment, but also to investigate whether this knowledge could contribute operationally to short-term predictions of recruitment in the ICES advisory system, we conducted several additional analyses. The annual stock assessment schedule for sprat in the Baltic is presented (Fig. 1).

Sprat is assessed in the annual April meeting of the Baltic Fisheries Assessment Working Group of ICES, of which the three authors of this paper are currently members. Recruitment and spawner biomass are estimated using an extended survivor analysis (XSA) applied to the years 1974–2005 for ages 1–8+ (Shepherd 1999; ICES 2006). The abundance of sprat recruits at age 1 is first estimated in internationally coordinated autumn acoustic surveys in the year of birth (peak spawning occurs in late April – early June, although in some years spawning is delayed or continues until July–August; Karasiova 2002). These survey estimates of recruitment are scaled to population level using a standard survey recruitment calibration program (Shepherd 1997) and the historic time series of survey-based and XSA-derived recruits.

In addition to these recruitment estimates, two additional recruitment estimates are needed for the short-term predictions. One estimate corresponds to year-class strength of the year in which the assessment is actually done; the second estimate is for the subsequent prediction year. Thus a total of three recruitment estimates are required for short-term predictions: YC_{t-1}, YC_t, YC_{t+1}, where t is the year when the assessment is conducted and YC designates the year class (Fig. 1). The survey-based recruitment estimates for YC_{t-1} are considered reliable because these indices have a high correlation ($R^2 \sim 0.9$) with the recruitment estimate from the XSA (ICES 2006). Recruitment for the current and subsequent year's year classes are estimated in the standard ICES procedure as the geometric mean (GM) recruitment for a period of years when recruitment fluctuated without trend or within a given regime (ICES 2006).

Environmentally based recruitment predictions can therefore potentially contribute to the recruit estimates for YC_t and YC_{t+1}. A forecast of recruitment for either of these year classes requires knowledge of the environmental conditions in sprat spawning and nursery areas for eggs, larvae, and age-0 groups. One of the main environmental variables that affects recruitment is spring–summer water temperature in these areas (Ojaveer 1998; MacKenzie and Köster 2004; Baumann et al. 2006). As it is not presently possible to predict reliably annual variations in these temperatures one year in advance, we focus on the prediction of recruitment for the current year class, YC_t, while applying the ICES procedure of using the GM of past recruitment to estimate recruitment for YC_{t+1}. Because of the timing of the assessment meeting,

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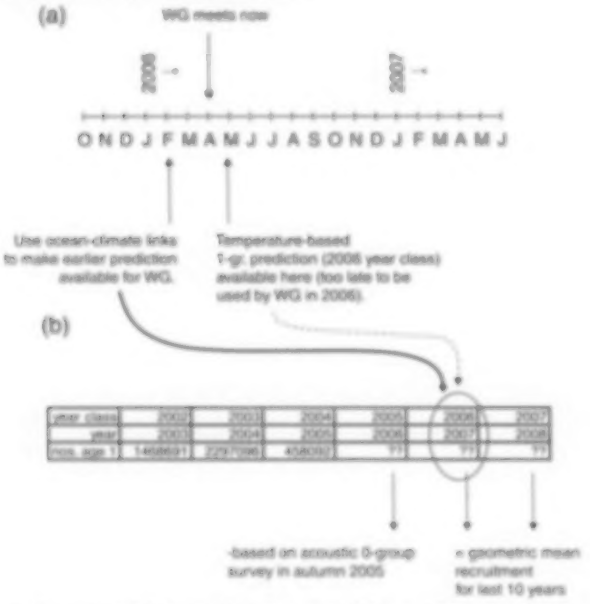
Fig. 1. (*a*) Schematic representation of the timing of the Baltic Fisheries Assessment Working Group (WG) relative to oceanographic variables that influence sprat (*Sprattus sprattus*) recruitment in the Baltic Sea (ICES subdivisions 22–32). Months are labelled by first letter only. In this example, the WG meets in April 2006 to assess stock status at the beginning of 2006 and to predict catch and stock development until the end of 2007 and spawner biomass at start of 2008. (*b*) The numbers at age 1 (denoted as 1-gr. in this figure) in the different years (ICES 2006) and the years for which year-class predictions are required for input to short-term predictions of catch and spawner biomass. In this study, we focus on how to use environmental variables to estimate the 2006 year class, which will be age-1 recruits in 2007.


(a)
WG meets now
2006
2007
O N D J F M A M J J A S O N D J F M A M J
Use ocean-climate links to make earlier prediction available for WG.
Temperature-based 1-gr. prediction (2006 year class) available here (too late to be used by WG in 2006).
(b)
year class
year
num. age 1
1468691
229?396
458092
-based on acoustic 0-group survey in autumn 2005
= geometric mean recruitment for last 10 years

environmental information that could be used by the Working Group must be available by April (Fig. 1).

Our earlier analyses (MacKenzie and Köster 2004) showed that temperature in May (i.e., the month corresponding to historic peak of spawning) explained significant variation in sprat recruitment and that May temperature can be predicted from both a winter (January–February) index of the North Atlantic Oscillation (NAO; Jones et al. 1997) and an ice coverage index of the Baltic Sea (Seinä and Palosuo 1993). As a result, both the NAO and the ice index were also significant correlates of sprat recruitment (MacKenzie and Köster 2004) and are potential candidates for operational incorporation into the sprat short-term prediction procedure. Therefore, our analyses in this report focus on the May temperature – NAO – ice cover interaction and how these variables affect sprat recruitment.

Updating of relationships among climatic and oceanographic variables and recruitment

Couplings between time series of hydroclimatic variables such as those used in this study can themselves vary over time (Jevrejeva 2002; Oguz et al. 2006). We first evaluate whether the relationships detected earlier between May temperature, the NAO, and ice coverage apply with the five additional years of data now available. A second series of analyses compares the sprat recruitment with these same environmental variables. These analyses employ scatterplots and linear regression modelling, including the estimation of 95% prediction limits for response variables (Sokal and Rohlf 1998).

Short-term predictions of sprat recruitment, spawner biomass, and landings

The predictions use numbers, weight, and maturity at age, as well as natural and relative fishing mortality at age as inputs. These data are provided as input to or output from the XSA, except for the numbers of recruits, which are provided by the acoustic survey for the previous year's year class or the GM recruitment for a multiyear period (1991–2005). The population data are projected forward assuming a range of user-defined levels of fishing mortality. Outputs are spawner biomass for the current and two subsequent years and landings for the current and one subsequent year. We conduct predictions of all three variables using the standard approach of ICES, including status quo fishing mortality, and by replacing the GM recruitment with an environmentally based prediction of recruitment for the current (assessment) year's year class. For operational predictive purposes, we only use the NAO because this variable is more likely to be available to the Working Group than the ice index (typically available in late March). The results do not differ substantially.

In addition, three further analyses were conducted to explore the range of possible spawner biomass and landings that could result depending on assumptions of environmental variability. One of these scenarios used a variance-weighted average of the mean log-transformed (ln) recruitment for the years 1991–2005 and the predicted ln recruitment from the regression relationship between ln recruitment and the January–February NAO (NAOJF). For this scenario and the first two scenarios described above, we estimated the 95% prediction intervals of the predicted recruitment; we subsequently used the prediction intervals for the recruitment to estimate the sensitivity of landings in 2007 and spawner biomass in 2007 and 2008 to recruitment uncertainty. The remaining two scenarios were exploratory and used the recruitment that could occur if the NAO in both 2006 and 2007 were the lower or upper 10th percentile of the NAOJF for the period 1973–2005. For illustrative purposes, we make predictions based on the sprat stock assessment conducted in spring 2006; the time horizon for predictions will cover the years 2006–2008.

Results

Relationships among climatic and oceanographic variables and recruitment

During the five years since the original analysis, the environmental variables have usually been at levels that characterize average or warmer-than-average conditions (Fig. 2). Recruitment has varied widely, with a very strong year class in 2003 (Fig. 2*d*). The relationships between the NAO, ice coverage, and May temperature observed earlier apply also for the five most recent years: most of the new years lie within the 95% prediction limits of the regression predictions based on the earlier time period (1973–1999; Fig. 3).

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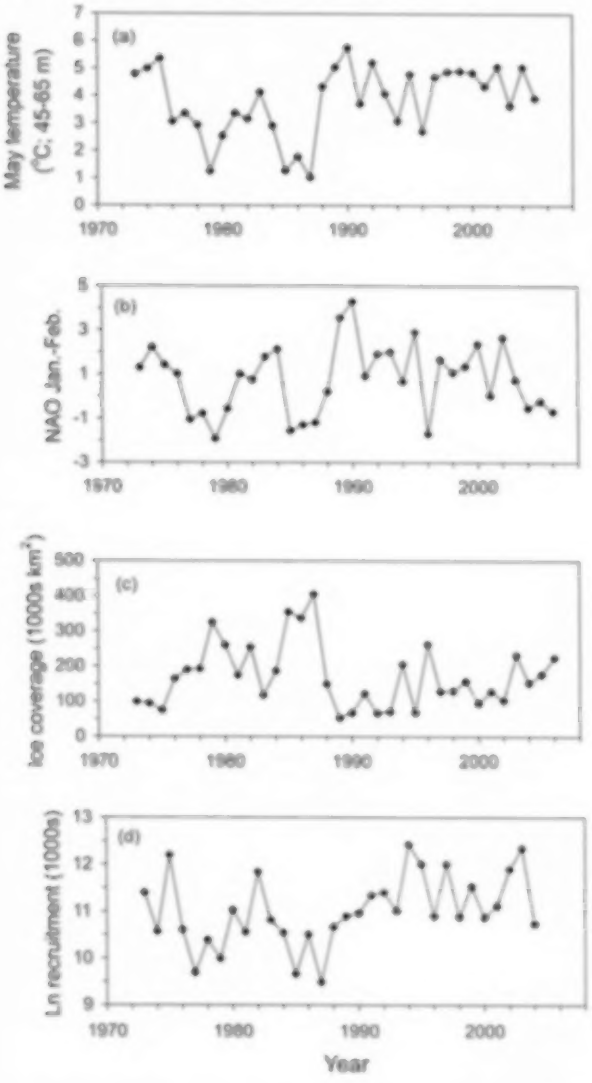
Fig. 2. Interannual variability in (*a*) May temperature at 45–65 m in the Bornholm Basin (Baltic Sea), (*b*) the North Atlantic Oscillation index (NAO; January–February), (*c*) maximal ice coverage of the Baltic Sea, and (*d*) recruitment (ln-transformed) of sprat in the Baltic Sea (ICES subdivisions 22–32; ICES 2006).

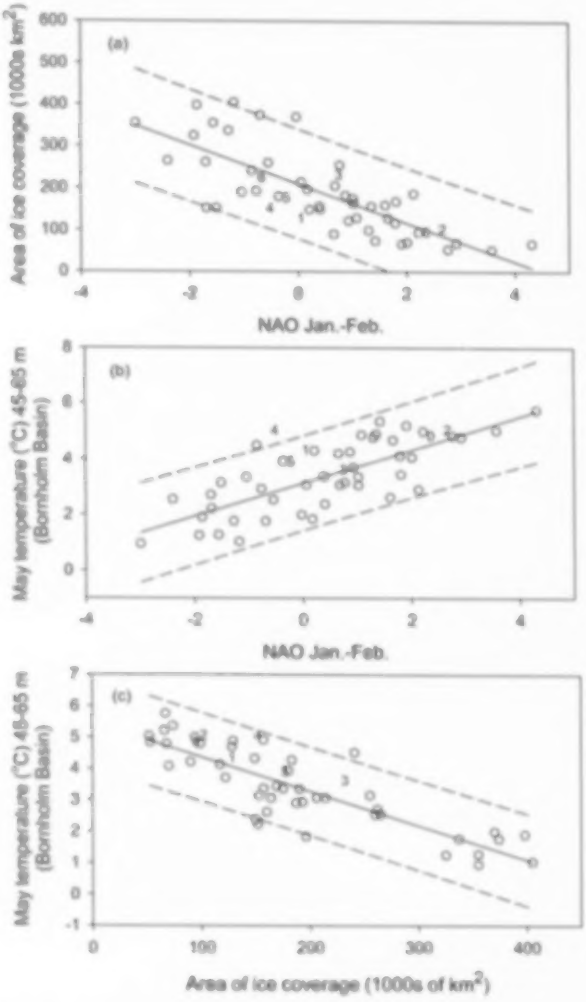


The five additional sprat year classes also depended on the three environmental variables consistent with the earlier relationships (Fig. 4). Four of the five year classes lie close to the regression prediction, whereas one year class (2003) is just outside the 95% prediction interval. The mean squared prediction error for the five new year classes, calculated as

$$\frac{1}{n}\sum(\ln\mathrm{OBS} - \ln\mathrm{PRED})^2$$

where OBS is observed recruitment and PRED is the recruitment predicted from the relationship between ln recruitment and NAO for the 1973–1999 year classes, was 0.526, and the root mean square error (RMSE) relative to ln-transformed mean predicted recruitment was 7%. A full retrospective analysis of forecasting ability of the environmental relationships for earlier year classes is available in MacKenzie and Köster (2004).

Fig. 3. Relationships between May temperature at 45–65 m in the Bornholm Basin (Baltic Sea), the North Atlantic Oscillation index (NAO; January–February), and maximal ice coverage of the Baltic Sea. The circles are data for 1973–1999 and the numbers represent years since 2000. The regression line (solid line) and 95% prediction intervals (broken lines) include the years 1973–1999 (MacKenzie and Köster 2004). The numbers represent years since 2000; these years were not included in the regression or prediction interval calculations.



Short-term predictions

We used the updated regression relationship between the NAOJF and ln recruitment (Fig. 4) to forecast recruitment for the 2006 year class. Recruitment was estimated to be 42 trillion (95% prediction interval (PI) = 10.1–177 trillion) based on this relationship and using the observed value of

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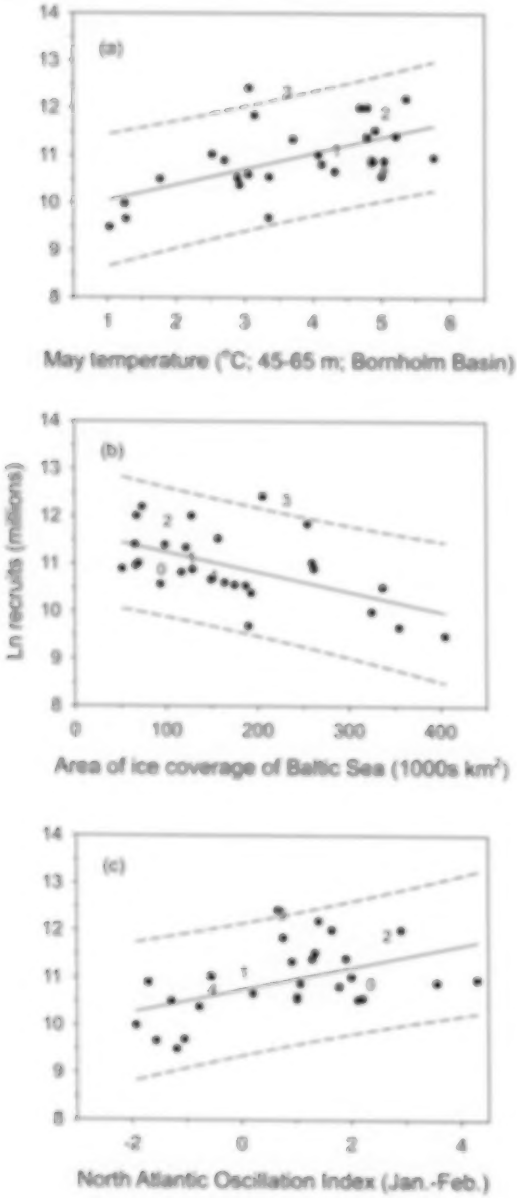
Fig. 4. Relationships between Baltic sprat (*Sprattus sprattus*) log-transformed recruitment and (*a*) May temperature at 45–65 m in the Bornholm Basin (Baltic Sea), (*b*) maximal ice coverage of the Baltic Sea, and (*c*) the North Atlantic Oscillation index (January–February; NAOJF). The circles are data for 1973–1999 and the numbers represent year classes since 2000. The regression line and 95% prediction intervals include the years 1973–1999 (MacKenzie and Köster 2004). Updated regression equations including all year classes (i.e., 1973–2004; $N = 32$) and related regression statistics are as follows: ln recruitment = 9.8 + 0.31*T* ($R^2_{adj} = 0.26$, $P = 0.0017$); ln recruitment = 11.6 – 0.0038 ice ($R^2_{adj} = 0.19$, $P = 0.0071$); ln recruitment = 10.8 + 0.23 NAOJF ($R^2_{adj} = 0.20$, $P = 0.0056$).



NAOJF for 2006 (–0.67). Alternatively, the predicted recruitment for the 2006 year class is 92 trillion (95% PI = 25.8–328 trillion) as estimated from the GM for the years 1991–2005 (Table 1). The variance-weighted average of these two values is 58 trillion (95% PI = 23.5–145 trillion).

The differences in recruitment influence spawner biomass in subsequent years (Table 1). Spawner biomass in 2007 is ca. 1.4 million tonnes regardless of which recruitment forecast is used (only 17% of sprat are, on average, mature at age 1; ICES 2006). However, in 2008, the predicted spawner biomass differs by a larger amount among the scenarios: assuming that recruitment is influenced by the NAO yields a spawner biomass 15% lower than the spawner biomass estimated using the GM recruitment for the 2006 year class.

The potential range of spawner biomass in 2008, given the lower and upper 10th percentiles for the historic range of the NAO (1973–2005) and other input assumptions, is 1.1–1.4 million tonnes (Table 1). The sensitivity of the spawner biomass two years beyond the current assessment year was found to be ~27%, given the range in NAO values used (10th–90th percentiles). The predicted landings in 2007 for the different options are very similar regardless of option used (373 000 – 390 000 t; Table 1).

Discussion

Extension of environment–recruitment relationships for sprat in the Baltic Sea

We have updated existing relationships between climatic and oceanographic variables and between recruitment and environment variables with five years of new data, or 24% of the original data series (1973–1999). The new data agree well with predictions made by the previous relationships and we conclude that in combination with results from MacKenzie and Köster (2004), temperature has positively influenced sprat recruitment for the last 50 years.

The positive influence of temperature on the Baltic sprat population is likely due to its northerly location relative to the range of the species as populations near the edges of their distribution tend to react more strongly to environmental perturbations than those near range centres (Frank 1991; Myers 1998). The only southern population for which recruitment estimates exist (Black Sea) responds negatively to temperature (Daskalov 1999); hence, the population-specific response of recruitment to temperature for sprat is dome-shaped and similar to that reported for Atlantic cod (*Gadus morhua*; Planque and Frédou 1999; Brander 2000).

The 2003 sprat year class was much stronger than that predicted from our environmental variables. We believe that this year class was strong because of exceptionally warm temperatures during the summer and especially the fall of 2002 and an average winter in 2002–2003. Also, temperature conditions in summer and fall 2003 in the Baltic Sea were nearly as warm as those in 2002 (MacKenzie and Schiedek 2007). The warm conditions in fall 2002 and 2003 (Mohrholz et al. 2006; MacKenzie and Schiedek 2007) would have stimulated sprat growth rates, maturation of juveniles, and egg production rates (Baumann et al. 2006; Reglero and Mosegaard 2006). Hence, although May temperature and the NAO were not too much higher than average in 2003, exceptional processes occurring later in

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Table 1. Basis for prediction of recruitment in 2006 and 2007 for five scenarios and predicted landings and spawner biomasses.

Scenario no.	Basis of 2006 recruitment prediction	Basis of 2007 recruitment prediction	Predicted recruitment for 2006 year class (billions)	Spawner biomass in 2007 (t)	Spawner biomass in 2008 (t)	Landings in 2007 (t)
1 (ICES standard run)	Geometric mean	Geometric mean	92 000 (26 000–328 000)	1 440 000 (1 400 000–1 600 000)	1 400 000 (1 080 000–2 360 000)	390 000 (371 000–460 000)
2	NAOJF – ln recruitment linear regression	Geometric mean	42 000 (10 000–177 000)	1 460 000 (1 380 000–1 500 000)	1 300 000 (1 010 000–1 720 000)	375 000 (366 000–416 000)
3	Variance weighted average of runs 1 and 2	Geometric mean	58 000 (23 000–145 000)	1 420 000 (1 400 000–1 490 000)	1 200 000 (1 070 000–1 580 000)	380 000 (370 000–406 000)
4	Lower 10th percentile of NAOJF during 1973–2005	Lower 10th percentile of NAOJF during 1973–2005	37 000	1 400 000	1 100 000	374 000
5	Upper 10th percentile of NAOJF during 1973–2005	Upper 10th percentile of NAOJF during 1973–2005	91 000	1 440 000	1 360 000	390 000

Note: For all scenarios, the recruitment for the 2005 year class was based on the standard ICES acoustic survey estimate. Geometric mean recruitment was based on year classes 1991–2005. Outputs for scenarios 1–3 include estimates using 95% prediction intervals of the 2006 recruitment as input to short-term predictions of landings and spawner biomass; the 95% prediction intervals quantify sensitivity of landings and spawner biomass to recruitment uncertainty. NAOJF, the North Atlantic Oscillation index (January–February).

the year, e.g., intense heating, summer inflows of warm water (Luterbacher et al. 2004; Mohrholz et al. 2006), may have promoted recruitment.

The exceptional heating of 2003 after an average winter demonstrates the potential consequences of environmental forecasting and its incorporation into fishery predictions. In this particular case, a larger than predicted year class was produced, probably because of the high temperatures in 2002 and later in 2003. In other years, an opposite event could happen, i.e., temperatures fail to rise after an average or mild winter, leading to unusually low recruitment. As a result, the criteria for deciding whether and when to include environmental information, when to stop using it, and how much weighting it should be given when making advisory decisions need consideration. Should a system be rejected because of one "bad" prediction among a specified number of years (e.g., 5 or 10)? What constitutes a bad prediction (e.g., an observation in the opposite direction of the prediction, an observation exceeding some predetermined margin of error even in the same direction of the prediction)?

These issues have received relatively little evaluation, at least within the ICES community with which we are most familiar. However, as the ecosystem approach to fisheries management develops and expands and as climate change progresses, these topics will require more attention in the future. One way forward would be to conduct a retrospective analysis of the performance of the environmental variables and compare this performance with a similar analysis using the standard method for making predictions. In the case of sprat in the Baltic Sea, the environmental predictions outperformed the existing methodology (MacKenzie and Köster 2004).

Implementing the environmental information could also proceed stepwise. For example, if it is not desirable to abandon the existing methodology, a combined approach based on a variance-weighted average of the different predictions made by different methodologies (e.g., geometric mean, regression models, artificial neural networks) could be developed. The performance of the standard, combined, and environment-only predictions could be monitored in the future until there is confidence that the environmentally based predictions are performing satisfactorily. As measuring platforms, oceanographic modelling capacities, and knowledge of recruitment processes improve, it is likely that recruitment predictions will also improve.

Short-term predictions for fisheries management

We have demonstrated how knowledge of recruitment processes, environmental information, and its uncertainty can be incorporated into the routine fishery advisory procedure for sprat in the Baltic Sea. In this specific case, there are peculiarities of the assessment calendar necessitating our search for and use of links between climatic and oceanographic variables to move the prediction horizon forward to meet the timing of the assessment meeting. This inevitably leads to a reduction in predictive ability because each additional link introduces additional unexplained variability. Nevertheless, the performance of these predictions is superior to that of the existing methodology (MacKenzie and Köster 2004).

For the predictions of landings in 2007 and spawner biomass in 2007 and 2008, we have demonstrated that both the

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landings and spawner biomass in 2007 are robust to the recruitment forecast for the 2006 year class. This is because the 1-year-olds generally contribute a relatively modest share of the catches and spawner biomass (17% of age-1 sprat are assumed to be mature; ICES 2006), although in some years, 50%–60% of age-1 sprat can be mature (ICES 2002). However, in 2008, when most (93%) of the 2006 year class is mature (ICES 2006), there are differences in spawner biomass. Given the NAO in 2006, the spawner biomass may be 15% lower than that currently estimated by ICES. Under the present relatively high stock level, a 15% overestimate of SSB may not be critical because the biomass is well above the critical biomass limit (B_{lim}) below which fishing mortality must be reduced. As a result, at the present time, a 15% difference in perception of the biomass is not as critical as if the biomass were at or below B_{lim}. In the present situation, therefore, the advice is not affected by the choice of the recruitment prediction methodology. However, if the spawner biomass were at or below B_{lim}, the fishery advice could depend on how the recruitment was estimated. In such a situation, the quality of predictions becomes more important and is a prerequisite if advice and management decisions are based on the best available evidence and knowledge.

The difference of 15% in spawner biomass estimates for 2008 is due to a single age group in a single year (i.e., the 2006 year class). Obviously, if a forecast of the subsequent year's recruitment (2007 year class) was possible, then forecasts of biomass and landings farther into the future could be made. However, the key environmental variables (e.g., May temperature, NAO) cannot presently be predicted with high confidence that far ahead (MacKenzie et al. 1996; Ulltang 2003). Moreover, time series approaches, which rely on autocorrelation in the recruitment data and which have been applied for recruitment forecasting in other populations (Ottersen and Sundby 1995; Needle et al. 2003; Planque et al. 2003), cannot be applied to sprat in the Baltic Sea because the autocorrelation in sprat recruitment is very low: autocorrelations at lags 1 and 2 were 0.27 and 0.3, respectively, and were lower at longer lags; none was significant ($P > 0.05$).

We note that surface temperatures and wind conditions later in the year (e.g., July–August) explain more recruitment variation than May temperatures (Baumann et al. 2006). However, predictions of surface temperatures and probably also wind conditions this late in the year from winter indices are presently more uncertain (MacKenzie et al. 1996; MacKenzie and Köster 2004; Hinrichsen et al. 2007). As a result, it is not yet possible to incorporate this information into the short-term prediction routine of ICES. This could be possible, however, if the timing of the assessment was shifted to later in the year or if processes affecting recruitment later in the year could be linked by modelling or statistical analysis to events earlier than when the Assessment Working Group meets.

ICES uses environmental information in short-term predictions of some other Baltic fish stocks (e.g., Gulf of Riga herring, *Clupea harengus*, recruitment is predicted using zooplankton abundance and sea temperature; Kornilovs 1995; ICES 2006). ICES has also included exploratory analyses of how temperature affects short-term predictions of Baltic sprat recruitment but has not yet adopted the environmentally based predictions in its advice (ICES 2006), which may be due to a reluctance to change from status quo methodology, particularly when the change would not influence advice to the fisheries. In recent years, there have been some other exploratory attempts to include environmental information in short-term recruitment predictions for other fish stocks. These include, for example, North Sea cod (*Gadus morhua*; Planque et al. 2003), Bay of Biscay anchovy (*Engraulis encrasicolus*; ICES 2006; Allain et al. 2007), and Northeast Arctic cod (*G. morhua*; Huse and Ottersen 2003; Svendsen 2006). However, none of these is operationally used in ICES assessments and work is still continuing to improve the recruitment prediction models and their implementation in assessment.

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Salmon carcasses as a marine-derived resource for benthic macroinvertebrates in a developing postglacial stream, Alaska

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Abstract: Streams formed in Glacier Bay, southeast Alaska, following glacial recession rapidly support populations of spawning salmon. Using both observational and experimental approaches, we examined the importance of pink salmon (*Oncorhynchus gorbuscha*) carcasses for benthic macroinvertebrate communities, approximately 10 years after salmon first colonized Wolf Point Creek, a first-order, lake-fed stream. Macroinvertebrate abundance on carcasses naturally accumulating in the stream channel was low, dominated by chironomids (*Eukiefferiella* spp.). Macroinvertebrate drift was significantly lower from artificial experimental channels containing salmon flesh ($P < 0.001$) compared with channels without. However, the density and community structure of macroinvertebrates colonizing rock baskets in channels was not significantly different between treatments. In a separate experiment, colonization of substrate trays with salmon and control (inert) fillets were associated with differences in community structure; simuliids dominated control fillets, and chironomids dominated fish fillets, while underlying stone substrate was colonized by a more diverse community including Ephemeroptera, Plecoptera, and Trichoptera taxa (EPT). Excluding simuliids, abundance was significantly higher on fish compared with control fillets ($P < 0.001$). These data suggest that scales and mucus prevent the direct consumption of flesh from whole carcasses, highlighting the role predators–scavengers in facilitating flesh consumption by benthic macroinvertebrates.

Résumé : Les cours d'eau qui se forment dans la baie Glacier dans le sud-est de l'Alaska à la suite du retrait des glaciers abritent très rapidement des populations reproductrices de saumons. À l'aide à la fois d'observations et d'expériences, nous avons examiné l'importance des carcasses de saumons roses (*Oncorhynchus gorbuscha*) pour les communautés benthiques de macroinvertébrés, environ 10 ans après que les saumons aient colonisé pour la première fois Wolf Point Creek, un cours d'eau d'ordre 1 alimenté par un lac. L'abondance des macroinvertébrés sur les carcasses qui s'accumulent naturellement dans le chenal du cours d'eau est faible et dominée par les chironomidés (*Eukiefferiella* spp.). La dérive des macroinvertébrés est significativement plus faible dans les canalisations expérimentales artificielles qui contiennent de la chair de saumon ($P < 0.001$) comparativement aux canalisations qui n'en ont pas. Cependant, la densité et la structure des communautés des macroinvertébrés qui colonisent des paniers de pierres dans les canalisations ne diffèrent pas en fonction du traitement expérimental. Dans une expérience séparée, la colonisation de plateaux de substrat contenant des filets de saumon ou des filets témoins inertes est associée à des différences de structure de communauté; les simuliidés dominent sur les filets témoins et les chironomidés sur les filets de poisson, alors que les substrats de pierres sous-jacent est colonisé par une communauté plus diversifiée comprenant des taxons d'éphéméroptères, de plécoptères et de trichoptères (EPT). Si on exclut les simuliidés, l'abondance est significativement plus élevée sur les filets de poisson par comparaison aux filets témoins ($P < 0.001$). Ces données laissent croire que les écailles et le mucus empêchent la consommation directe de la chair sur les carcasses entières, ce qui met en lumière le rôle des prédateurs et des nécrophages qui facilitent la consommation de la chair par les macroinvertébrés benthiques.

[Traduit par la Rédaction]

Introduction

Quantifying the input and passage of energy and nutrients through ecosystems presents a major challenge to contemporary ecology (Polis et al. 2004; Belgrano et al. 2005). Despite the longstanding recognition of marine subsidies to freshwater ecosystems (Juday et al. 1932; Shuman 1950), the paradigm of marine-derived nutrients (MDN) as a driver

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in freshwater ecosystems is comparatively recent (see reviews by Kline et al. 1997, Gende et al. 2002, and Naiman et al. 2002). Nonetheless, the perceived importance of MDN is reflected by the classification of Pacific salmon (*Oncorhynchus* spp.) as a "keystone species" in the ecosystems of the American northwest (Willson and Halupka 1995; Willson et al. 1998).

The role of macroinvertebrates in processing heterotrophic resources has been highlighted as an important functional process facilitating the incorporation of allochthonous inputs (e.g., Petersen and Cummins 1974; Wipfli et al. 1998). The comparatively high nutrient content of returning salmon (Shearer et al. 1994; Gende et al. 2004) provides a rich resource for any organism able to exploit it (Quinn et al. 2003; Johnston et al. 2004; Walter et al. 2006). Delivered to lotic ecosystems with seasonal predictability, the arrival of spawning salmon in freshwater ecosystems punctuates a critical life-cycle cue for a variety of animals (Quinn and Buck 2000; Winder et al. 2005; Hocking and Reimchen 2006) and may act as an important driver in the phenology of macroinvertebrate life histories (Moore et al. 2004). Representing an alternative food resource for lotic macroinvertebrates, it has been suggested that the presence of salmon carcasses may account for the contrasting community structure of salmonid streams in south-central Alaska that support a higher proportion of filter feeders than streams without salmon (Kline et al. 1997).

Stable isotope analysis provides quantitative evidence of the uptake of marine-derived N and C in epilithic organic matter, benthic macroinvertebrates, juvenile fish, and riparian vegetation (e.g., Bilby et al. 1996; Helfield and Naiman 2002; Claeson et al. 2006). However, a comprehensive understanding of the importance of salmon-derived resources is limited by the lack of information on the mechanisms of nutrient and energy transfer (Gende et al. 2002). Despite the abundant evidence for the direct consumption of carcasses, the information available on scavenging by macroinvertebrates remains ambiguous (Lessard and Merritt 2006). Nevertheless, carcass use by a diversity of macroinvertebrate fauna has been documented (e.g., Chaloner and Wipfli 2002; Minakawa et al. 2002; Winder et al. 2005). Direct sampling of macroinvertebrates from whole carcasses has furnished knowledge of taxa that exploit carcasses in natural systems (e.g., Fenoglio et al. 2005). However, it often remains unclear whether carcasses are providing food or habitat; particulate organic matter becomes entrained in the fungal matt of carcasses (Minshall et al. 1991), and stranded carcasses retain other allochthonous food that is consumed by macroinvertebrates (Flory and Milner 1999).

Knowledge of carcass use through direct and indirect pathways by benthic macroinvertebrates is compounded by characteristics of this dynamic resource. Artificial channels have provided an important means of experimental reductionism; however, logistics and costs restrict the scope of an exhaustive replicated design, with observational records representing an important supplement to explain experimental results (e.g., Wipfli et al. 1998). Typically colonized by drifting fauna, artificial channels limit the species pool of potential colonists with potentially important scavengers, such as caddisfly larvae that are largely absent (Wipfli et al. 1998). Surveying above and below impassable falls has provided corroborative evidence at the whole-stream scale; however, the complex variability of the river habitat and the effects of redd disturbance have thwarted an unambiguous interpretation of the relative importance of carcass enrichment per se (Chaloner et al. 2004). Furthermore distinguishing between direct and indirect use is not trivial; exudates from carcasses enrich organic biofilms, stimulate microbial activity, and increase primary production downstream of stranded carcasses (Richey et al. 1975; Bilby et al. 1996; Johnston et al. 2004), complicating the investigation of spatially discrete substrates (i.e., defining adjacent substrate that is or is not affected by carcass decay), emphasising the need for the design of complementary experiments and their subsequent mutual interpretation to build a more comprehensive understanding of salmon carcasses as a macroinvertebrate resource.

While the populations of Pacific salmon have declined in many regions (NRC 1996), salmon runs in Alaska and parts of Canada remain close to their historic abundance (Baker et al. 1996; Slaney et al. 1996). The high-latitude ecosystems of the Pacific Northwest have therefore played a seminal role in the study of MDN, where salmon carcasses continue to exercise an important influence on recipient macroinvertebrate communities (e.g., Kline et al. 1997; Wold and Hershey 1999; Chaloner et al. 2002). Recently deglaciated streams in southeast Alaska represent an ecologically important subset of these ecosystems, providing unique information on the dynamics of lotic succession at the catchment scale (e.g., Milner 1987; Flory and Milner 2000; Milner and York 2001). These recently formed streams are typically colonized by salmon after a few decades (Milner and Bailey 1989), with evidence from stable isotope analysis indicating that MDN play an increasingly important role as stream age increases (Milner et al. 2000). Knowledge of the processes that determine the integration of MDN into lotic ecosystems as succession proceeds provides important understanding to the application of carcasses as a tool for river restoration and to understanding the mechanisms that drive lotic succession generally.

The spawning run of comparatively young streams can be considerable (Monaghan and Milner 2008), indicating that the apparently limited uptake of MDN in younger streams is not related to resource supply. However, these developing streams tend to lack course woody debris and pool habitat (Milner et al. 2000), providing comparatively poor conditions for the in-stream retention of salmon carcasses, which may limit the availability of this resource to the relatively short period of the spawning run (Monaghan and Milner 2008). Furthermore, the concomitant construction of salmon redds during this period is often associated with reduced invertebrate densities (Monaghan and Milner 2008; Moore et al. 2004; Walter et al. 2006), potentially compromising the ability for resource exploitation by this otherwise important consumer community.

With a fixed 2-year life cycle, populations of pink salmon in Glacier Bay streams alternate between years of high abundance (odd years) and low abundance (even years). In association with manipulative experiments, we exploited this natural variation in carcass resources and macroinvertebrate abundance to test the importance of salmon flesh as a resource for the benthic macroinvertebrate community of a

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developing stream, approximately 10 years after pink salmon first colonized it. Specifically, our hypotheses were to (*i*) document the rate of flesh decay in an evolving stream environment; (*ii*) distinguish between salmon flesh as a food or habitat; and (*iii*) elucidate the short-term relevance of adjacent benthic substrate in the exploitation of salmon MND.

Materials and methods

Study area

The study was undertaken in Wolf Point Creek, Glacier Bay National Park, southeast Alaska (Fig. 1), a postglacial stream that formed approximately 50 years prior to this study (Milner 1994). The first-order stream is lake-fed, cutting through a bedrock gorge with a fall of approximately 30 m, providing a barrier to fish migration before flowing for approximately 2 km in an easterly direction over unconsolidated glacial moraine, till, and outwash deposits with a low gradient to the ocean. During the period of this study, stream substrate was dominated by large cobbles (10–20 cm), with catchment vegetation characteristic of an early-succession plant community, with mountain alder (*Alnus viridis* ssp. *crispa*) and willow (*Salix* spp.) dominating riparian vegetation.

Fish and invertebrate communities

The invertebrate community of Wolf Point Creek has been dominated by chironomids since its initial colonization, with detailed descriptions of successional change and community organisation documented in a number of descriptive and experimental studies (Milner 1987, 1994; Flory and Milner 2000). Records for fish colonization date from 1988 for Dolly Varden (*Salvelinus malma*), 1989 for pink salmon (*Oncorhynchus gorbuscha*), 1990 for coho (*Oncorhynchus kisutch*), and 1996 for chum salmon (*Oncorhynchus keta*) and slimy sculpin (*Cottus cognatus*) (Milner 1994; Monaghan 2002). During the summer field seasons of this study, the spawning run was dominated by pink salmon, with foot surveys conducted between July and August estimating an escapement of approximately 10 000 adult returns in 1997 and 2000 for the same period in 1998.

Carcasses in the natural stream channel

Attempts to describe the use of whole tethered carcasses in the natural system during periods of no or low spawning were abandoned because of interference from wildlife (brown bear (*Ursus arctos*), weasel (*Mustela* sp.), and wolverine (*Gulo gulo*)). However, during a period (August 1997) of high natural abundance of carcasses and live salmon, interference from scavenging mammals was negligible, permitting repeat observations of macroinvertebrate taxa present on the same carcasses at different stages of decay.

Experimental methods

Artificial channels and colonization trays were employed to investigate the importance of salmon flesh for the macroinvertebrate community under manipulated conditions. For both experiments, the carcasses of spent pink salmon were collected in late August 1997 from Wolf Point Creek. Collections were restricted to carcasses with no visible signs of decay (e.g., fungal growth). Carcasses were cut into boneless fillets (four from each fish) and frozen for use the following year (1998).

Fig. 1. Map of Muir Inlet in Glacier Bay National Park (Alaska, USA), showing Wolf Point Creek and the site's glacial history.

In situ colonization

Substrate collected from a dry stream channel was washed and separated into three approximate size classes (2–5 cm; 1–2 cm; <1 cm diameter). Wooden trays (30 cm × 22 cm; area 0.066 m^2) were filled with stones from the two larger size classes, and a handful of the smallest stones (to fill interstices) were secured to the streambed. Each tray had one salmon fillet secured to the stone-covered surface. To provide a reference, inert as food resource but simulating the physical character of a fish fillet (cf. real leaf packs vs. plastic leaf packs; Flory and Milner 1999; Negishi and Richardson 2006), control fillets were fabricated from cardboard wrapped in insulating tape to prevent disintegration. Wire loops were attached to all fillets and used to fasten fillets to their respective trays and facilitate rapid sampling, minimizing disturbance to macroinvertebrate colonists. Salmon fillets (each representing the flesh from one-quarter of a whole fish) were weighed before being fastened to substrate trays and reweighed on removal to estimate mass loss.

The arrangement of colonization trays on the streambed posed ambivalent logistical objectives. Experimental design in patch-scale colonization experiments must address the spatial and temporal heterogeneity in abiotic conditions and organism distribution (Hurlbert 1984) and complex ecological issues such as temporal variability in organism mobility

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(Winterbottom et al. 1997), the dynamic qualitative characteristics of patch resources (Boyero et al. 2006), and the disturbance effects associated with sample removal. Existing knowledge of invertebrate use of salmon carcasses presented several caveats for experimental design. Given the potential for successional change and sequential resource processing (Elliott 1997), the overall design was based on simultaneous placement and sequential removal. Based on the rapid colonization of fish carcasses and patch-scale substratum (Mackey 1992; Kline et al. 1997; Wipfli et al. 1998), removal was planned for days 1, 3, 6, 10, 15, 21, and 28 after initial tray placement. Trays for removal on the same day were arranged in blocks to minimize disturbance (of remaining replicates) associated with tray removal. Finally, because macroinvertebrate use of dead salmon has been strongly associated with exudates downstream of carcasses (e.g., Richey et al. 1975; Schuldt and Hershey 1995; Bilby et al. 1996), a nonrandomized, serial placement was employed to avoid potential contamination of control treatments by exudates from fish treatment trays with replicate treatments aligned in downstream series (parallel to the flow). While this design sacrificed the randomization of any unknown spatial differences at the block scale, the experiment was conducted within an environmentally homogenous stretch of the stream. Testing this assumption in ecological terms (from Surber samples collected from respective fish and no-fish sides of the block) revealed no systematic difference amongst the 12 most numerous macroinvertebrate taxa between the location of fish verses control treatments ($N = 8$; one-way analysis of variance, ANOVA; $P > 0.25$). Rows of trays within each experimental block were separated by approximately 50 cm; columns, representing fish and control treatments, were 70 cm apart. All blocks were separated by at least 5 m. Depth and current velocity were measured at each replicate unit.

Trays within blocks were removed from the stream in a sequential, upstream direction to avoid disturbance to remaining sample units. Each fillet was removed from its tray and placed in a sealable plastic sac containing alcohol (to kill macroinvertebrates). Stone substrate and associated macroinvertebrates were similarly emptied into a separate sealable plastic sac. Macroinvertebrates were separated from mineral and organic substrate and preserved in 70% alcohol prior to identification. Daily inspection of the tray experiment revealed that after 19 days all salmon fillets had disappeared. On day 19, plastic fillets were removed from all remaining trays for consistency in comparative (physical) conditions in the control treatment.

Experimental channels

Eight artificial channels, 2 m long and 18 cm wide, were fed with stream water and drifting macroinvertebrates from the stream channel. Each channel was filled with substrate from the stream, simulating natural benthic conditions. Replicate rock baskets (volume 1.0×10^{-3} m^3), three per channel, were positioned in parallel fashion in each channel. Channels were randomly divided into fish and no-fish treatments. Eight fillets (equating to the flesh from two pink salmon) were placed at the top of each fish-treatment channel such that water flowing through the channel passed over the fish flesh. A wire cage structure, designed to exclude scavengers, was nailed down over the upper portion of the channels that housed the salmon flesh. Drift nets (250 μm) were placed over channel exits to collect macroinvertebrates exiting channels in diurnal and nocturnal drift samples; all drift sampling was conducted for a minimum of 7 h. One rock basket from each of the eight channels were sampled 7, 14, and 21 days after the placement of salmon flesh.

Statistical analysis

Mass loss of salmon flesh (not corrected for any mass loss in alcohol) was converted to percentages and described by the best-fit function according to least-squares regression. Macroinvertebrate population data were converted to natural log (ln) abundance. Population data from the tray colonization experiment were analysed by two-way analysis of covariance using the general linear model. Fish and days since placement represented fixed factors, with downstream sequential tray position, depth, and mean current velocity (measured independently at each tray site) as covariates. Pairwise post hoc comparisons (Tukey–Kramer range test) were used to identify significant differences between respective treatments. Population data were analysed at the community level and, for macroinvertebrates that were numerically abundant, at the individual level. Macroinvertebrate populations on stone and fillet substrates were described collectively and separately to illustrate differences between substrate types from fish and control treatments. Data from artificial channels were analysed by one-way ANOVA using post hoc comparison to test for significant differences between sampling dates in the rock basket experiment.

Results

Descriptive observations

Carcasses were rapidly colonized by a grey microbial matt, typically 3–4 mm thick and becoming increasingly dense over time. Fine particulate matter became entrained in the fungal matt. Very few macroinvertebrates were observed on carcasses. Average populations on carcasses ranged between 10 and 30 macroinvertebrates per carcass, regardless of the time available for colonization. Macroinvertebrates present on carcasses were dominated by *Eukiefferiella minor* and *Eukiefferiella claripennis*. Almost all muscle tissue had decayed after approximately 4 weeks, leaving the skin and bones intact.

Colonization trays

Linear decay of salmon fillets occurred rapidly, with no flesh after 21 days ($r^2 = 0.98$; Fig. 2). Total macroinvertebrate abundance on colonizing trays increased over time ($F_{[56,6]} = 9.965$; $P < 0.001$), although this general trend was interrupted by a decrease in abundance with the absence of fillets (both fish and control) on samples collected on day 21 (Fig. 3). Overall abundance was not significantly different between treatments (Fig. 3); covariates were not significant. Taxon richness increased significantly over time ($F_{[56,6]} = 35.31$; $P < 0.001$), but was not significantly different between fish and control trays, although mean richness was consistently higher by one–two taxa compared with control treatments (Fig. 4).

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Fig. 2. Mean percentage of fillet remaining (by mass) on substrate trays planted in the stream channel on consecutive sampling occasions. Error bars represent 1 standard error.

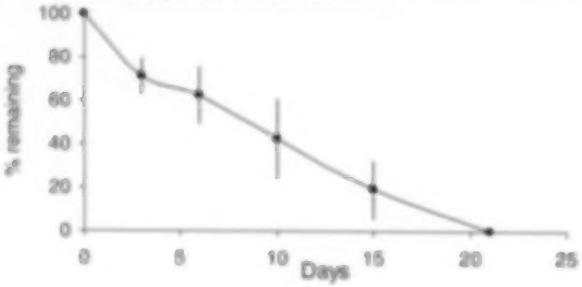
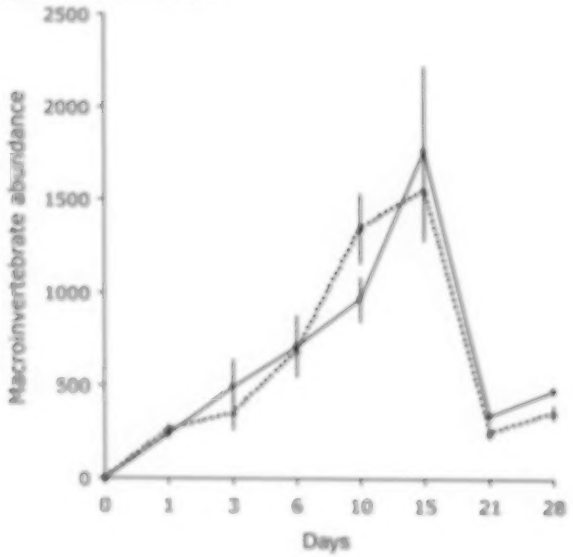


Fig. 3. Mean macroinvertebrate abundance colonizing implanted trays, with fish (solid line) and control (broken line) fillets, described in relation to days since tray placement in stream. Plastic fillets were removed from control trays on day 19. Error bars represent 1 standard error.



For the five sampling occasions that fillets were present (days 1–15, inclusive), taxonomic richness on both fish and control fillets increased over time ($F_{[4,1]} = 52.08$; $P < 0.05$) and was consistently higher on fish compared with control fillets ($F_{[1,1]} = 52.08$; $P < 0.001$; Fig. 5). Taxonomic richness was higher on stone substrate compared with fillet substrate for both fish and control treatments (Fig. 5). These generalized patterns reflect the contrasting community structure among control fillets dominated by simuliids, fish fillets dominated by chironomids, and stone substrate represented by a more diverse community including simulids; chironomids; and Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa (primarily Baetidae, Chloroperlidae, and Limnephilidae; Figs. 6*a*–6*d*).

Excluding simuliids from analysis revealed the increase in macroinvertebrate abundance was significantly higher on fish compared with control treatments (fish: $F_{[6,1]} = 109.91$; $P < 0.001$; pairwise comparisons: $P < 0.05$). By day 15, overall abundance was 2.5 times higher in fish compared with control treatment ($F_{[3,1]} = 33.65$; $P < 0.001$) and 14 times higher on fish fillets compared with control fillets ($F_{[3,1]} = 45.97$; $P < 0.001$), reflecting corresponding differences in the abundance of several individual taxa (Table 1). Filter-feeding simuliids were, in turn, significantly higher on control fillets compared with fish fillets; populations were

Fig. 4. Mean taxon richness of macroinvertebrates colonizing implanted trays with fish (solid line) and control (broken line) fillets over time. Error bars represent 1 standard error.

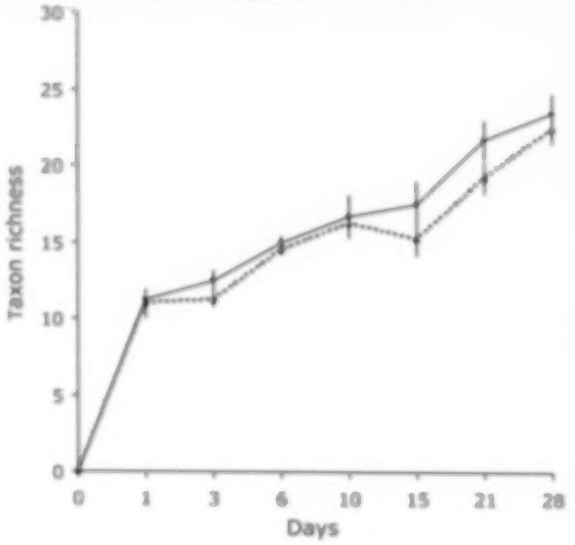


Fig. 5. Mean taxon richness of macroinvertebrates colonizing fillets and stone substrates by treatment: control fillet (lower broken line), fish fillet (lower solid line); stone substrate, control treatment (upper broken line); stone substrate, fish treatment (upper solid line). Error bars represent 1 standard error.

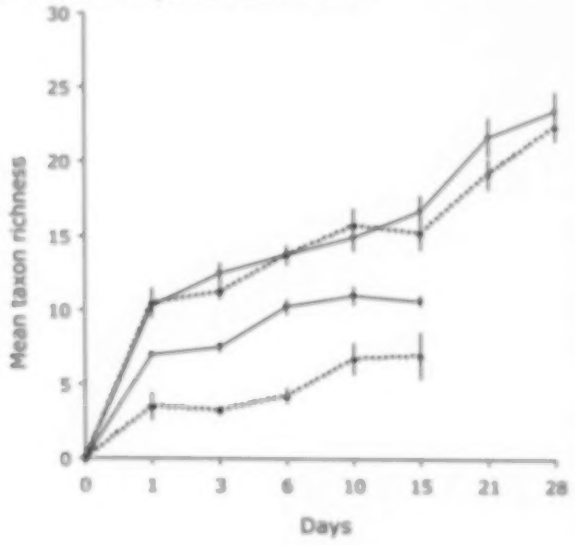


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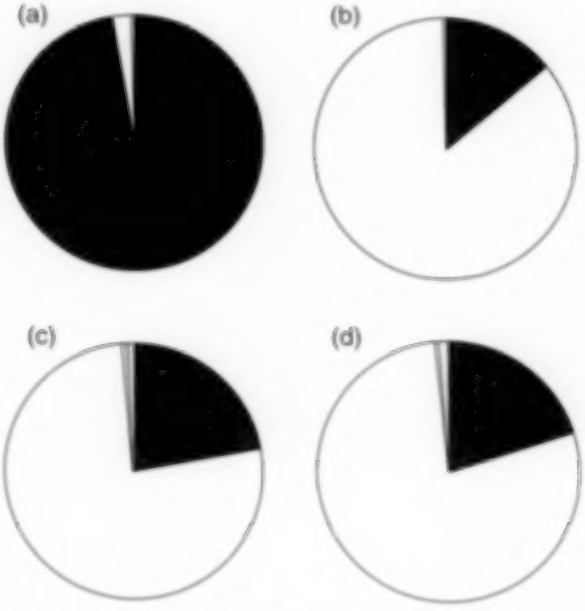
Table 1. Mean macroinvertebrate abundance for selected taxa colonizing respective substrate types 15 days after placement in the stream channel.

	Fillets			Stones		
Taxa	Fish	Control	Fish vs. control	Fish	Control	Fish vs. control
Pagastia partica	5.75 (2.63)	1.75 (0.85)	NS	13.00 (6.06)	12.75 (7.68)	NS
Eukiefferiella minor	14.25 (2.78)	3.25 (1.97)	°	7.50 (2.87)	10.5 (2.72)	NS
Orthocladius (*Eudactylocladius*) sp.	157.0 (21.8)	14.25 (7.43)	°	110.3 (12.7)	96.8 (16.0)	NS
Eukiefferiella gracei	141.0 (37.3)	4.5 (2.96)	°°	85.0 (12.7)	54.0 (8.54)	NS
Eukiefferiella claripennis	285.5 (22.1)	16.25 (10.7)	°°°	63.8 (22.5)	56.5 (10.7)	NS
Simulium sp.	15.5 (1.0)	293.0 (55.2)	°°°	993.0 (285.0)	964.0 (703.0)	NS
Baetis sp.	1.25 (0.25)	0.0	°°°	4.75 (1.75)	3.75 (1.5)	NS
Sweltsa fiorinata	0.0	0.0	—	2.25 (0.48)	0.5 (0.29)	*
Oligochaeta	1.0 (0.41)	1.25 (0.48)	NS	5.5 (2.33)	5.0 (1.68)	NS
Ecclisomyia sp.	0.0	0.0	—	2.0 (0.41)	1.0 (0.71)	NS

Note: Mean values are given, with standard error in parenthesis. Summarized results of one-way analysis of variance (ANOVA) are provided: NS, nonsignificant; °, $P < 0.05$; °°, $P < 0.01$; °°°, $P < 0.001$.

Fig. 6. Contrasting community structure on respective substrate types on day 15: (*a*) fish fillet; (*b*) control fillet; (*c*) stone substrate, fish treatment; (*d*) stone substrate, control treatment. Macroinvertebrate populations: chironomids (black); simuliids (open); Ephemeroptera, Plecoptera, and Trichoptera taxa (EPT) (grey); other (stippled).

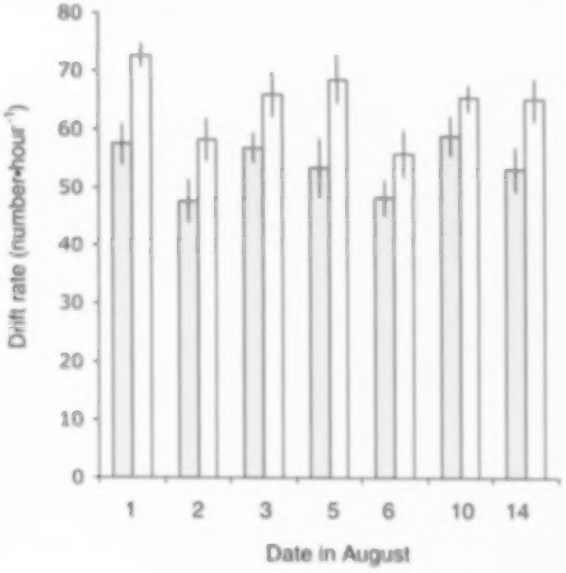


not significantly different on stone substrates from respective fillet treatments (Table 1).

Fish channels

Diurnal drift samples revealed low drift rates with no overall significant difference in macroinvertebrate drift from channels with fish flesh or reference channels. Nocturnal samples revealed drift rates, on average, 2.4 times higher than diurnal rates, with the drift rate consistently higher from reference channels ($F_{[1,54]} = 26.34$; $P > 0.001$; Fig. 7), indicating more macroinvertebrates remained in channels with fish flesh. These community-level patterns were reflected by different drift rates for several individual taxa (Table 2). Invertebrate densities in rock baskets increased significantly over time ($F_{[1,2]} = 9.01$; $P < 0.01$). However, neither total macroinvertebrate abundance nor the abundance of any individual taxa was significantly different between fish and reference channels on any sampling occasion.

Fig. 7. Mean nocturnal drift rates from artificial channels containing salmon flesh (shaded) and no salmon flesh (open). Error bars represent 1 standard error.



Discussion

The rapid decay of fish flesh was probably due to a combination of leaching, abrasion, and biological processing. The linear decay suggests that these degrading processes remained constant throughout the 15+ day period that the fillets were present on implanted trays. In other studies, the

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Table 2. Comparison of mean nocturnal drift rates from artificial channels containing salmon flesh (F) and no salmon (R, reference).

	Sampling dates (August)						
Taxa	1st	2nd	3rd	5th	6th	10th	14th
Orthocladius (*Eudactylocladius*) sp.	R > F**	R > F	R > F	R > F	R > F	R < F	R > F
Eukiefferiella gracei	R > F*	R > F	R > F*	R > F*	R > F	R > F	R > F**
Eukiefferiella claripennis	R > F*	R > F	R > F	R > F	R > F*	R > F	R > F*
Simulium sp.	R > F	R > F*	—	R > F	—	R < F	R > F
Total abundance	R > F*	R > F	R > F*	R > F*	R > F	R > F	R > F*

Note: Dash indicates insufficient abundance for statistical analysis. All other taxa were not significantly different throughout the experiment. *, $P < 0.05$; **, $P < 0.01$.

decay rates of whole carcasses have been best described by an exponential function, with rapid processing of flesh followed by the slower decomposition of scale and bone material (Minshall et al. 1991; Parmenter and Lamarra 1991; Fenoglio et al. 2005). Despite the difference in partial and whole carcasses, decay proceeded considerably faster in this study compared with another study in southeast Alaska, where carcasses lost 60% of their weight after 90 days (Wipfli et al. 1998) at water temperatures between 7 and 8 °C. In Wolf Point Creek, temperatures were warmer, ranging between 12.2 and 17.0 °C (Monaghan 2002), and the decay rate more closely resembled the decay rate in a Mediterranean stream where Fenoglio et al. (2005) documented a weight loss of >75% over 27 days.

The results of the manipulative experiments indicate that fish flesh was an important resource for some macroinvertebrates. Several species showed selective colonization, with simuliids significantly more abundant on control fillets and several chironomid species more numerous on fish fillets. Simuliids proclivity for smooth substrate as a site of attachment, as provided by the plastic fillets, has been widely documented (Ciborowski and Clifford 1984; Hershey and Hiltner 1988; Dudley 1992). In contrast, the comparatively slimy surface of the fish skin or the spongy texture of the exposed flesh, in addition to the developing microbial matt on salmon fillets, probably offered an unfavourable habitat for the attachment of their basal silk pads. Despite the potential importance of textural differences, this issue has received little attention in other comparative resource studies (e.g., terrestrial leaves versus plastic "leaves"; Graça and Pereira 1995; Flory and Milner 1999), and its potential confounding influence in investigations of nutritional value is difficult to evaluate. Nonetheless, sustaining an increasingly large population of macroinvertebrates over time means the presence of adequate food resources can be inferred regardless of textural differences at the substrate surface. However, whether the food consumed can be attributed to the fish flesh or from a differential capacity of fillets to trap organic particles from the water column is more difficult to elucidate. Evidence from observational surveys, where there was no increase in invertebrate abundance on naturally accumulating carcasses over time, suggests that the exposed flesh was the decisive factor.

Discounting the filter-feeding simuliids that are independent of nutrient resources on the substrate itself, macroinvertebrate abundance was higher on fish compared with control fillets. This observation is particularly emphatic given the smaller surface area of fillets compared with that of the underlying stone substrate, indicating their strong preferential colonization. While fish fillets had a higher profile on the stream bed and, therefore, may have intercepted more drifting macroinvertebrates than the underlying stone substrate, this does not account for the difference between fish and plastic fillets that approximated the same physical form. It is instructive in this respect to note that the abundance of macroinvertebrates on stone substrate was consistently higher on fish treatments compared with control treatments, although differences were not significant. As the texture of these two habitats were identical, it suggests that particles of fish abraded from the fillets, and (or) dissolved nutrients sorbed onto substrate surfaces (Bilby et al. 1996), provided the only consistent difference between these habitats. Thus, the importance of different nutritional value in the colonization of these substrates and, by extension, of salmon flesh and (or) associated exudates in the local downstream environment is indicated (Wipfli et al. 1998; Minakawa et al. 2002; Claeson et al. 2006).

Despite the diversity of macroinvertebrates that have been found in association with dead salmon and their range of feeding strategies and mouthpart morphology, few studies have reported a succession of macroinvertebrates processing fish carcasses (Kline et al. 1997; Wipfli et al. 1998; Fenoglio et al. 2005). While the study of Elliott (1997) provides an exception to this pattern, it is notable that his experiment was based on juvenile dead fish, as opposed to adults, presenting a contrasting packaging of this potentially nutritious resource. Nonetheless, apparent sarcophagous feeding by macroinvertebrates has been widely reported (e.g., Brusven and Scoggan 1969; Chaloner and Wipfli 2002; Minakawa et al. 2002). Although limnephilid caddisflies are numerous in the margin habitats of Wolf Point Creek (Flory and Milner 1999), the invertebrate community is dominated by chironomids, which were the only family significantly associated with salmon fillets in the tray placement experiment. Their higher abundance on flesh compared with control fillets and greater densities on stone substrate from fish treatments suggests that larvae were feeding directly on carcass flesh, although unequivocal proof would require an analysis of ingested material, which was not conducted in this study.

Contrasting with evidence from our manipulative experiments, but corresponding with results from research elsewhere, whole carcasses naturally accumulating in Wolf

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Point Creek were sparsely populated by macroinvertebrates (cf. Minshall et al. 1991; Schuldt and Hershey 1995; Claeson et al. 2006). Differing slightly from this general pattern, Fenoglio et al. (2005) reported comparatively high numbers of macroinvertebrates on the carcasses of rainbow trout (*Oncorhynchus mykiss*); however, total abundance was low in comparison with densities on adjacent benthic substratum and did not increase over time. In general, salmon appear to be colonized soon after death, often by relatively high numbers of macroinvertebrates, but are subsequently abandoned, with microbial processing dominating the biological breakdown of whole carcasses while macroinvertebrates play a negligible role (Minshall et al. 1991; Kline et al. 1997; Claeson et al. 2006), with increased macroinvertebrate productivity overwhelmingly associated with indirect uptake (Wipfli et al. 1998, 1999). Observing a similar fate for alewife (*Alosa pseudoharengus*) carcasses in a coastal Atlantic stream, Garman (1992) applied knowledge of decomposition processes in terrestrial systems and suggested that aquatic microbes, competing for limited resources (i.e., carcasses), may produce toxic compounds that render the resource unviable for other organisms and preclude invertebrate colonization. The apparently contradictory evidence from this study suggests a possible alternative explanation. While the colonization tray experiment demonstrated that carcass flesh was an important food resource, whole carcasses were not. The difference in the presentation of these otherwise similar resources is difficult to ignore; the fillet experiment presented a large area of exposed flesh, whereas whole carcasses presented a surface covered by scales and mucus. Thus, the fish skin and scales appear to have acted as a protective barrier, preventing macroinvertebrates from accessing the nutritious muscle tissue and precluding the direct use of carcasses as a food resource. Winder et al. (2005) similarly observed caddisfly larvae preferentially colonizing salmon carcasses mutilated by bear, suggesting this selective behaviour might be explained in terms of facilitation, permitting access past the otherwise tough skin of adult salmon. The patchy and dynamic distribution of carcasses, their high probability of stranding outside the aquatic environment, and their comparatively ephemeral residence in developing streams such as Wolf Point Creek (Monaghan and Milner 2008) indicates that adaptive radiation to exploit this otherwise nutritious resource may not represent a viable evolutionary response for the macroinvertebrate communities of comparatively young streams.

Over the time scale of this study, there was no evidence of macroinvertebrate productivity being enhanced via the indirect route in either manipulative or descriptive studies. While macroinvertebrate densities in the current study were higher on stone substrate from fish treatments as opposed to plastic treatments in the tray experiment, densities in rock baskets were not significantly different, suggesting that the differences probably resulted from the consumption of abraded flesh (i.e., direct as opposed to indirect consumption of MDN). In the stream channel itself, the density of benthic macroinvertebrates and benthic organic matter (excluding carcasses) remained very low because of the continued presence of spawning adults and associated redd disturbance (Monaghan 2002). These findings contrast with other similar research. Wipfli et al. (1999) reported a significant increase in chlorophyll *a* in the presence of decaying carcasses after 15 days, with levels remaining between two and eight times higher than reference channels throughout the 90-day experiment. Observed increases in autotrophic production were accompanied by a similarly rapid response by secondary consumers. In a comparison of stream reaches that were accessible or not accessible to spawning fish, higher levels of primary productivity was observed in salmon reaches but no overall difference in macroinvertebrate biomass, revealing a complex response at the individual (genus) level (Chaloner et al. 2004).

The speculation of Kline et al. (1997) that rivers with spawning salmon might be reflected in an increased proportion of filter feeders was not supported by this investigation, which was designed to assess salmon carcasses as an invertebrate resource. The only known filter feeders (*Simulium* spp.) clearly avoided the flesh itself and were no more abundant in potentially enriched areas downstream of decaying flesh. Interestingly, a more generally descriptive study, concurrent with this research, found the relative abundance of *Simulium* spp. were increased in the main channel, although this was attributed to the mechanical disturbance process of redd excavation and the resultant reduction in benthic organic material that provided a favourable physical habitat (Monaghan 2002). In terms of trophic resources, this change in community structure appeared to be related to a depletion of benthic food and, as such, was independent of salmon carcasses.

Considering the evidence from this study in the context of other experimental research leads us to concur with the conclusion of Chaloner et al. (2004) that the environmental characteristics of the particular stream are likely to play a major role in the effects of semelparous, anadromous fish by defining the extent of carcass retention, the rate of decay, the relative importance of additional nutrient inputs, and the response of the lotic community. While the importance of salmon flesh was clearly demonstrated, the direct use of whole carcasses that naturally accumulated in the channel was not evident in this comparatively young stream. However, the apparent consumption of exposed and abraded flesh conforms to expectations based on relative nutritional value and supports the potential role of bears and other large predators in facilitating the exploitation of salmon carcasses by a wider stream fauna (Winder et al. 2005). From the perspective of stream restoration, data from this early successional community indicate that the macroinvertebrate uptake of MDN via indirect pathways may require the long-term retention of carcasses.

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Long-term shifts in the species composition of a coastal fish community

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Abstract: To study decadal shifts in a coastal nekton community, we analyzed data on 25 fish and invertebrate species collected from 1959 to 2005 by the University of Rhode Island, Graduate School of Oceanography (Narragansett, Rhode Island, USA). This weekly trawl survey samples two locations: inside Narragansett Bay and in Rhode Island Sound. Over four decades, the community has shifted progressively from vertebrates to invertebrates and, especially since 1980, from benthic to pelagic species. Demersal species that declined include winter flounder (*Pseudopleuronectes americanus*), silver hake (*Merluccius bilinearis*), and red hake (*Urophycis chuss*); meanwhile warm-water fish (butterfish, *Peprilus triacanthus*; scup, *Stenotomus chrysops*) and invertebrates (lobster, crab, squid) increased with time. Total numbers reached a maximum in the 1990s, while mean body size decreased. Taxonomic diversity increased over time, as the community shifted from fish to invertebrates of several phyla. The shifts in species composition correlate most strongly with spring–summer sea surface temperature, which increased 1.6 °C over the 47-year time series. Species composition was also correlated with the winter North Atlantic Oscillation index and chlorophyll concentration, which has declined since the 1970s. Triggered primarily by rising temperatures, these decadal changes have altered the trophic structure of the nekton community, resulting in a shift from benthic to pelagic consumers.

Résumé : Afin d'étudier les changements dans la communauté du necton côtier à l'échelle des décennies, nous analysons les données sur 25 espèces de poissons et d'invertébrés récoltées de 1959 à 2005 par le Graduate School of Oceanography de l'University of Rhode Island (Narragansett Bay, Rhode Island, USA). Cet inventaire hebdomadaire au chalut échantillonne deux sites, l'intérieur de la baie de Narragansett et le détroit du Rhode Island. Au cours des quatre décennies, la communauté s'est modifiée graduellement d'une dominance de vertébrés à une d'invertébrés et, particulièrement depuis 1980, d'espèces benthiques à pélagiques. Les espèces démersales qui ont connu un déclin incluent la plie rouge (*Pseudopleuronectes americanus*), le merlu argenté (*Merluccius bilinearis*) et la merluche rouge (*Urophycis chuss*); en revanche, les poissons d'eau chaude (le stromatée à fossettes, *Peprilus triacanthus*; le spare doré, *Stenotomus chrysops*) et les invertébrés (homards, crabes, calmars) se sont accrus dans le temps. Les nombres totaux ont atteint un maximum durant les années 1990, alors que la taille corporelle moyenne diminuait. La diversité taxonomique a augmenté avec le temps, à mesure que la communauté changeait des poissons aux invertébrés appartenant à plusieurs phylums. La corrélation la plus forte s'observe entre les changements de composition spécifique et la température de la surface de la mer au printemps et en été qui s'est accrue de 1,6 °C au cours de la période de 47 années. Il y a aussi une corrélation entre la composition spécifique et l'indice de l'oscillation de l'Atlantique Nord en hiver et la concentration de chlorophylle, en déclin depuis les années 1970. Occasionnés principalement par l'augmentation des températures, ces changements à l'échelle des décennies ont modifié la structure trophique de la communauté du necton, ce qui a entraîné le remplacement des consommateurs benthiques par des consommateurs pélagiques.

[Traduit par la Rédaction]

Introduction

Fish populations vary on temporal scales ranging from interannual to millennial (Hilborn and Walters 1992; Baumgartner et al. 1992). In most biotic time series with durations in the range of 30–50 years, variability increases with the number of years included in the calculation, resulting in "reddened" power spectra (Pimm and Redfern 1988). This decadal variability manifests itself as strings of years with high and low abundances, with important implications for population persistence, human governance, and decision-making. Interannual recruitment variability can be filtered or damped by the adult biomass of iteroparous fish species with long life spans (Spencer and Collie 1997). In contrast, decadal-scale variability squarely fits the life spans of many fish species and human planning horizons (Steele 1996).

Decadal variability can arise from a number of processes: species interactions, delayed density dependence, and spatially explicit or age-structured population dynamics (Akçakaya et al. 2003). Decadal-scale population variability can also be inherited from environmental variations, which may be quasi-cyclic (Chavez et al. 2003) or secular trends. Non-

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linear species interactions can amplify decadal variability in climatic forcing, resulting in abrupt and large-amplitude shifts in the biota (Collie et al. 2004). Such regime shifts in community composition create additional challenges to understanding population variability and for informing human decisions (Scheffer et al. 2001).

The long-term data that exist for freshwater and marine communities reflect the interplay of these population processes. Eutrophication has shifted many lakes from clear-water states to persistent turbid-water states with concomitant effects on the food web (Carpenter 2003). Dramatic changes in community structure of the Laurentian Great Lakes have been driven by phosphorous loading and trophic interactions among a succession of introduced species (Bunnell et al. 2006).

The best time series of marine fish abundance come from trawl surveys on the continental shelves. In the northwest Atlantic, North Sea, and Gulf of Thailand, dramatic shifts in species composition have occurred, in response primarily to fishing pressure and secondarily to changes in the marine climate (Hall 1999). On the eastern Scotian Shelf, commercial fishing and decadal variability in water temperature appear to have shifted the fish community from a demersal- to a pelagic-dominated state (Choi et al. 2004). The existing examples of species replacements on decadal time scales suggest some form of compensation mediated by competition and predator–prey interactions (Hall 1999). For example, the depletion of cod stocks in the North Atlantic over the past 40 years has been correlated with increased abundance of shrimp, crab, and lobster (Worm and Myers 2003).

Changes in community composition have also been observed in estuarine fish assemblages, although the existing data are often discontinuous and nonstandardized (Attrill and Power 2002; Genner et al. 2004). Compared with those on the continental shelf, estuarine fish communities may have greater exposure to anthropogenic stresses. They are more accessible to fisheries and may also suffer greater levels of pollution, habitat loss, and the presence of more introduced species. For example, estuarine species are exposed to endocrine disruptors that can alter sex ratios (Beresford et al. 2004). With shallower water columns, estuaries may respond more quickly to climate change than deeper waters offshore.

The University of Rhode Island, Graduate School of Oceanography (GSO; Narragansett, Rhode Island, USA), fish trawl is one of the few long-term, standardized fish surveys. Conducted weekly since 1959, the fish trawl has documented decadal shifts in the fish community of a temperate estuary (Jeffries and Johnson 1974). Several hypotheses have been advanced to explain the replacement of resident demersal fish species with seasonal migrants and invertebrate species (Jeffries and Terceiro 1985). One hypothesis is the sequential fishing of resident bay species favoring the offshore migrant species as local resources were depleted (Oviatt et al. 2003). The expected consequences of fishing are the removal of predator species (Jackson et al. 2001) followed by shifts in size structure to smaller forage species as a result of predator release (Myers et al. 2007). A shift from demersal to pelagic species is expected in eutrophied estuaries, as has been observed in European semi-enclosed seas (de Leiva Moreno et al. 2000). Finally, rising water temperatures (Oviatt 2004) should favor southern over boreal species. This study documents the long-term patterns in the GSO fish trawl and examines the utility of several community metrics for tracking the large shifts in species composition. We examine which forcing variables (fishing, chlorophyll, temperature, and other climate variables) are related to the shifts in species composition and the derived community metrics. The choice of forcing variables is limited to those with time series of sufficiently long duration. Finally, we examine the support for the various hypotheses that have been advanced to explain the shifts in species composition.

Materials and methods

Field

Every week since 1959, the demersal community at two stations in Narragansett Bay, Rhode Island, has been sampled by the GSO fish trawl. One station is located mid-bay in the West Passage, east of Fox Island, at a depth of 7.3 m; the second is located in Rhode Island Sound running south of Whale Rock at a depth of 20 m (Fig. 1). In this paper, we use "bay" to refer to the Fox Island station and "sound" to refer to the Whale Rock station. In the history of the GSO fish trawl, four vessels have been used: F/V *Billie II* (13 January 1959 – 28 June 1977), F/V *Dulcinea* (28 June 1977 – 16 July 1982), F/V *Gail Ann* (16 July 1982 – 8 September 1987), and R/V *Cap'n Bert* (8 September 1987 – present). Surface and bottom water temperatures were recorded at each station with a standard Niskin bottle and bucket thermometer.

The trawl nets, deployment, and towing speed have been standardized throughout the time series. The otter trawl has a bottom sweep of 15.2 m, an effective opening of 6.5 m, and cod-end mesh size of 5.1 cm (Captain Tom Puckett, University of Rhode Island, Kingston, RI 02882, USA, personal communication). The trawl doors, attached 18.2 m from the net mouth, are 1.52 × 0.76 m. Winches let out a marked amount of cable adjusted to the depths of each station: 36.5 m of cable at Fox Island and 82 m at Whale Rock. Tow duration at each station is 30 min at a speed of 2 knots (3.7 km·h^{-1}) over bottom. Following each tow, the net is retrieved and the contents are emptied into a checker box, sorted to the lowest taxon possible, counted, and weighed. The weight data began in 1994 and therefore are not used in this study.

Analyses

Of 130 species recorded in the GSO trawl-survey database, the most numerous 25 species from the years 1959–2005 were selected for this analysis (Table 1). This subset was selected to ensure that all 25 species were sufficiently numerous at both stations to have been identified consistently since the start of the fish trawl. Though the abundance of particular species has changed markedly, their frequency of occurrence has been consistent over time. These top 25 species accounted for 96% of a total of over 1.8 million animals recorded; therefore their dynamics will adequately capture the main shifts in community composition. Sampling was missed in occasional weeks because of poor weather or boat maintenance. Of 564 months, all but one had two or

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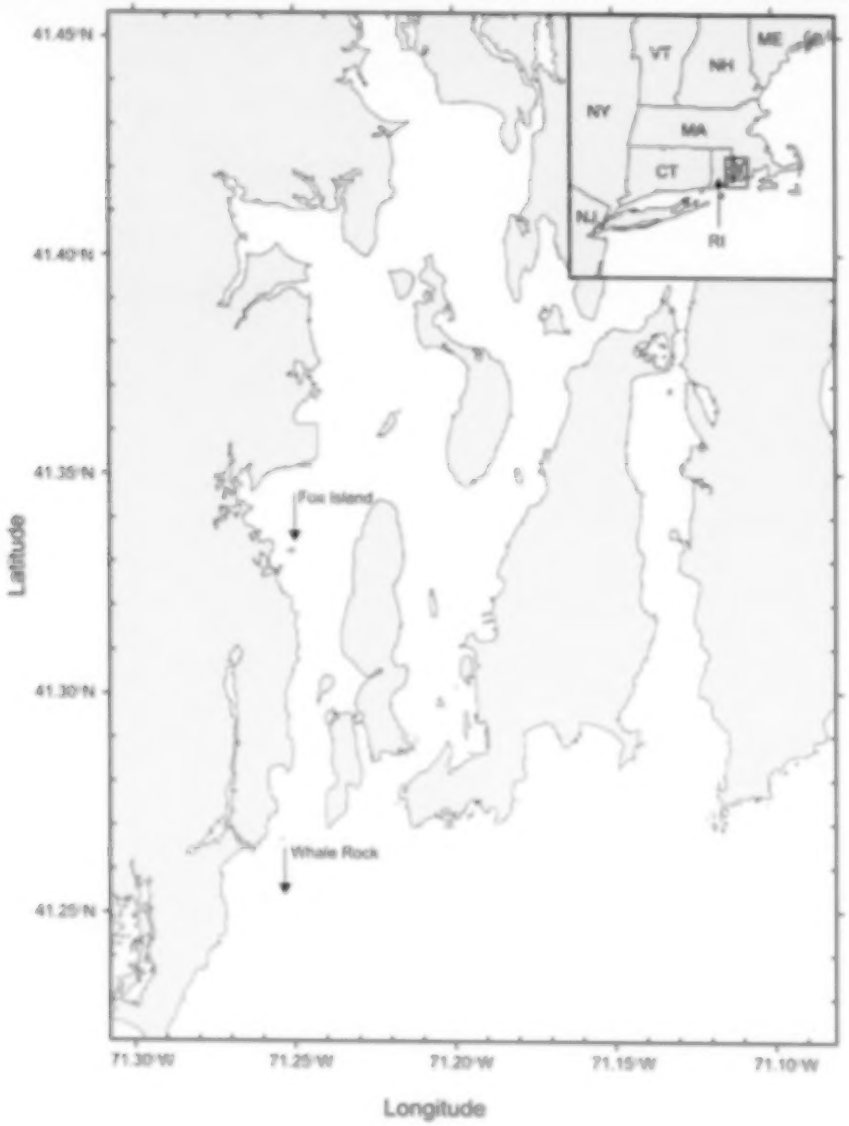
Fig. 1. Map of Narragansett Bay (Rhode Island, USA) showing the two bottom-trawl stations. The bay station is north of Fox Island and the sound station is south of Whale Rock. Inset shows location in the northeastern USA. Arrows indicate the direction and distance of each tow.



more surveys; 91% of the months had three or more surveys. To minimize the potential effect of missing data, we first calculated monthly means and then averaged the monthly means to obtain the annual mean catch per 30-min tow of each species.

Ordination of the species abundances by year was based on the Bray–Curtis similarity index (*S*) calculated from square-root-transformed annual mean abundance. The Bray–Curtis measure is widely used and has properties that are desirable for ecological studies, including coincidence, complementarity, relative invariance, independence of joint absence, localization, and dependence on totals (Clarke et al. 2006). Nonmetric multidimensional scaling (MDS; Field et al. 1982) was applied to the similarity matrices to ordinate the years in two dimensions. Similarity matrices were compared with the Spearman rank correlation coefficient (ρ). We tested for serial shifts in species composition by correlating the similarity matrix of the samples with a similarity matrix constructed from the rank-ordered years (Clarke et al. 1993).

A stepwise algorithm was used to identify sets of species that accounted for most of the temporal pattern of the community composition. Starting with a random subset of species, the BVSTEP routine sequentially adds and subtracts species (Clarke and Warwick 1998). The test statistic is the rank correlation between the similarity matrices for the sub-

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Table 1. Top 25 species in the University of Rhode Island, Graduate School of Oceanography, trawl survey from 1959–2005.

Common name	Scientific name	Habitat	Maximum size (cm)	Preferred temperature (°C)	Mean catch per tow
Scup	*Stenotomus chrysops*	Demersal	40.0	14.5	71.25
Winter flounder	*Pseudopleuronectes americanus*	Demersal	46.0	8.8	65.62
Cancer crab (Atlantic rock and Jonah)	*Cancer irroratus* and *Cancer borealis*	Demersal	14.0[a]	14.5	60.61
Butterfish	*Peprilus triacanthus*	Pelagic	30.5	13.0	49.06
Silver hake	*Merluccius bilinearis*	Demersal	76.0	10.0	33.88
Longfin squid	*Loligo pealeii*	Pelagic	40.0	12.0	28.97
American lobster	*Homarus americanus*	Demersal	80.0	11.0	12.73
Little skate	*Leucoraja erinacea*	Demersal	54.0	8.5	11.32
Windowpane	*Scophthalmus aquosus*	Demersal	51.0	13.4	11.02
Red hake	*Urophycis chuss*	Demersal	50.0	8.5	9.53
Spider crab	*Libinia emarginata*	Demersal	10.0[a]	11.5	5.78
Atlantic herring	*Clupea harengus*	Pelagic	43.0	7.0	4.99
Northern searobin	*Prionotus carolinus*	Demersal	45.0	16.0	4.98
Sea star	*Asterias forbesi*	Demersal	12.5[b]	22.5	4.94
Longhorn sculpin	*Myoxocephalus octodecemspinosus*	Demersal	45.7	9.3	4.80
Fourspot flounder	*Paralichthys oblongus*	Demersal	45.0	11.4	3.70
River herring (alewife and blueback herring)	*Alosa pseudoharengus* and *Alosa aestivalis*	Pelagic	37.0	9.0	3.09
Cunner	*Tautogolabrus adspersus*	Demersal	43.2	14.5	1.87
Summer flounder	*Paralichthys dentatus*	Demersal	61.0	15.5	1.82
Lady crab	*Ovalipes ocellatus*	Demersal	7.5[a]	13.0	1.38
Horseshoe crab	*Limulus polyphemus*	Demersal	60.0	30.0	1.29
Bluefish	*Pomatomus saltatrix*	Pelagic	115.0	13.5	1.10
Striped searobin	*Prionotus evolans*	Demersal	48.5	14.7	0.87
Tautog	*Tautoga onitus*	Demersal	90.0	13.5	0.81
Weakfish	*Cynoscion regalis*	Pelagic	100.0	21.8	0.79

Note: Maximum size is total length, unless otherwise indicated. Mean catch per tow was calculated over both stations and all years.

[a] Carapace width.

[b] Arm length.

set of species and the similarity matrix of the full community. Though different subsets may give the same correlation (redundant species), by repeated runs, a set of species was identified that was consistently correlated with the full community.

Trends in the abundance of individual species were measured by regressing the logarithm of abundance against time ($\ln N_t = \ln N_0 + mt$). Years with zero catches for particular species were replaced with one-half the minimum catch for that species. Across all species, the incidence of zero catches was 7.1% at the bay station and 8.9% at the sound station. The slopes of these semilogarithmic regressions (m) and their associated confidence intervals were used to indicate the temporal trends in species abundance.

We calculated the standard species diversity indices (Simpson, Shannon–Weiner, Pielou) and a diversity index that accounts for the taxonomic relatedness of the species, which were classified by species, genus, family, order, class, and phylum (Supplemental Table S1, available online from the NRC Data Depository).[2]

Taxonomic distinctness (Δ^+) is an extension of the Simpson index, which accounts for the taxonomic distance (ω_{ij}) between species i and j, while removing the dominating effect of the species abundance distribution ($x_i x_j$):

$$\Delta^+ = \frac{\sum\sum_{i<j} \omega_{ij} x_i x_j}{\sum\sum_{i<j} x_i x_j}$$

Taxonomic diversity measures have been found to be more sensitive to environmental change and less sensitive to sample size than traditional diversity indices (Warwick and Clarke 1995). Taxonomic distinctness is expected to decrease with increasing disturbance. A spatial analysis of trawl-survey data in the North Sea found that areas with low taxonomic distinctness corresponded with low abundance of elasmobranches, a taxonomic group the life-history characteristics of which make it particularly sensitive to fishing (Rogers et al. 1999).

The pelagic–demersal ratio was calculated simply as the sum of individuals of pelagic species divided by the sum of individuals of demersal species (de Leiva Moreno et al. 2000). Each species was assigned a maximum size, L_{max},

[2] Supplementary data for this article are available on the journal Web site (http://cjfas.nrc.ca) or may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Building M-55, 1200 Montreal Road, Ottawa, ON K1A 0R6, Canada. DUD 3756. For more information on obtaining material refer to http://cisti-icist.nrc-cnrc.gc.ca/cms/unpub_e.html.

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from the literature (Table 1). Mean L_{max} across species, weighted by the yearly mean abundance of each species, was used as a proxy for mean size in the community because length and weight measurements were not made in all years. We also defined a preferred temperature (T_{pref}) for each species as the midpoint of the preferred temperature range taken from the literature (Table 1). Mean T_{pref}, weighted by the annual mean abundance of each species, was use to index the temperature preference of the fish community over time.

A set of environmental data was selected to correlate with the species abundance data summarized by the diagonal matrix of Bray–Curtis dissimilarity (D), which is the complement of similarity ($D = 1 - S$). This set was limited to variables with sufficiently long time series to permit meaningful tests. We used the sea surface temperatures (SST) measured on the fish trawl because of a gap in bottom temperatures between 1964 and 1977. Monthly mean temperatures were calculated, again to minimize potential effects of missing weeks. The monthly means were then averaged to obtain seasonal and annual means. Chlorophyll *a* concentration was measured from 1973–1990 and 1999–2005 in Narragansett Bay near our Fox Island station (Fulweiler et al. 2007). The chlorophyll concentrations are annual means of weekly samples from the surface and bottom of the water column. Trawl fishing effort data from the fishing area that includes Rhode Island Sound (Northeast Fisheries Science Center (NEFSC) statistical area 529) were used as an index of fishing pressure. These nominal effort data are in units of days fished per year for the period 1965–2005.

In addition to these local variables, we examined potential correlations with basin-scale climate indices. Calculated from the difference in normalized sea-level pressure between the Azores and Iceland, the North Atlantic Oscillation (NAO) is an index of wind patterns over the North Atlantic (Hurrell 1995). The winter NAO index (December through March) is the dominant climate signal in the North Atlantic (Hurrell 1995) and has been widely related to biotic indices (e.g., Stenseth et al. 2003), including estuarine fish populations (Attrill and Power 2002). The Atlantic Multidecadal Oscillation (AMO) is the area-weighted average SST in the North Atlantic (Enfield et al. 2001).

The fish trawl catches individuals starting at about 10 cm long and 1 year old. Hypothesizing that fish abundance may be related to past environmental conditions, we calculated correlations with the environmental variables at lags of 0, 1, and 2 years. We used the BIOENV procedure, which performs a nonparametric Mantel test (Clarke and Ainsworth 1993). Rank correlation coefficients were calculated between the matrix of environmental variables (Euclidean distance) and the matrix of community data (Bray–Curtis dissimilarity). Randomization tests were used to test the significance of the correlations. Recognizing that autocorrelation in the time series and multiple testing increase the chance of spurious correlations, we used $p = 0.01$ as a critical level.

In addition to the multivariate correlations, we conducted univariate linear regressions of the biotic (dependent) variables against potential explanatory (independent) variables. The biotic variables included the grouped species abundances (squids, pelagic fish, benthic invertebrates, and demersal fish) and several community metrics (taxonomic distinctness, pelagic–demersal ratio, and mean L_{max}). Three of the environmental variables (annual SST, winter NAO, and the AMO) were available for all 47 years. These three variables were entered into a stepwise regression, with minimum Akaike information criterion (AIC) as the criterion for inclusion. For the two variables that were not available for the full time series, days fished and chlorophyll *a*, we used simple linear regression with the probability of a nonzero slope as the measure of significance.

Results

The species composition of the fish and invertebrate community has changed dramatically since 1959 (Fig. 2). Following a decade of dominance by demersal fish in the 1960s, benthic invertebrates became more abundant in the 1970s, especially at the sound station (Fig. 2*b*). The main shift was in the rise of the pelagic fish and squid in the 1980s, such that pelagic species accounted for more than half of the numbers in 1994. This shift to pelagic species has persisted since 1995, though demersal fish have regained dominance at the bay station (Fig. 2*a*).

Ordination of the community data by nonmetric, multidimensional scaling (MDS) revealed significant serial shifts in species composition at both stations (Fig. 3). The points cluster by decade, especially at the sound station (Fig. 3*b*). There was a significant correlation between the similarity matrices at each site ($\rho = 0.79$, $p = 0.001$), indicating a shared temporal pattern, though the species composition and community trajectories differed somewhat between stations. The major shifts occurred between 1984 and 1985 at the bay station and between 1980 and 1981 at the sound station (Fig. 3). There is no indication that species composition in the 2000s is returning to the community that characterized the start of the time series in 1959. Between 2002 and 2003, there was a suggestion at the bay station that the community might be shifting toward the species composition that prevailed in the 1970s (Fig. 3*a*). On the other hand, the tight clustering of the points since 1995 suggests that the community at the sound station has shifted to a persistent configuration.

Most of the 25 species had significant trends in abundance during the 47-year period (Fig. 4). To put these trends in perspective, an instantaneous rate of -0.1-year^{-1} sustained for 47 years would leave approximately 1% of the initial population. Cunner, longhorn sculpin, northern searobin, silver hake, and winter flounder had similar rates of decline at both stations. On the other hand, striped searobin, fourspot flounder, river herring, bluefish, butterfish, scup, little skate, *Cancer* crab, lady crab, American lobster, and longfin squid all had significant rates of increase at both stations (see Table 1 for species names). Atlantic herring was the only species with no significant temporal trend at either station (Fig. 4). The instantaneous rate of increase or decrease (m) is a useful way to summarize the time trends, but the actual trajectories are irregular, not smooth exponential curves, as shown below.

At each station, BVSTEP was used to identify a subset of species that accounted for most of the temporal pattern in community composition that was illustrated by the ordina-

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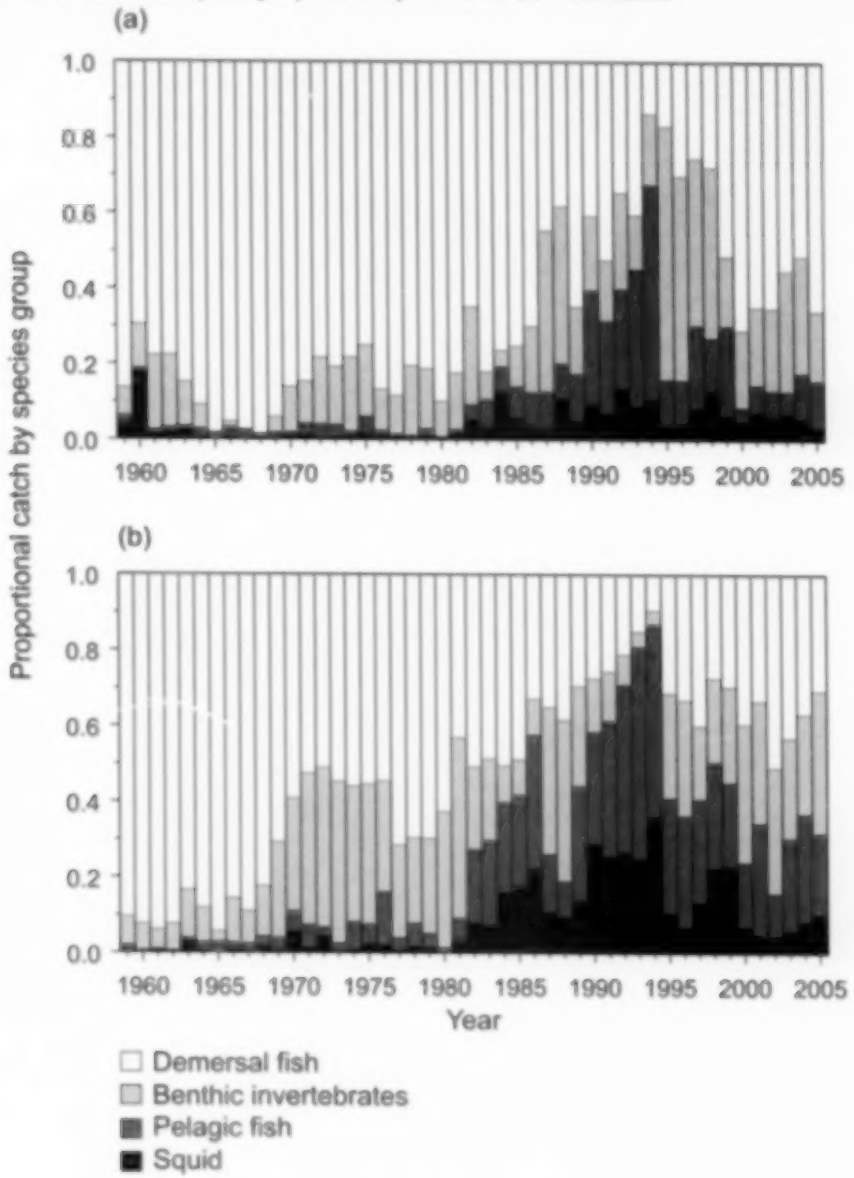
Fig. 2. Shifts in the relative abundance of species groups at (*a*) Bay Station and (*b*) Sound Station.



tion of the similarity matrices for all 25 species (Fig. 3). The similarity matrix of the abundances of these species over years had a rank correlation (ρ) of 0.95 with the corresponding similarity matrix for all 25 species. At the bay station, this subset comprised nine species: three demersal fish that decreased over time (winter flounder and silver and red hake), two fish (butterfish and scup), one elasmobranch (little skate), and three invertebrates (*Cancer* crab, American lobster, and longfin squid), which all increased (Fig. 5). Together, these nine species accounted for 58% of the dissimilarity between the two clusters of years in Fig. 3*a*, as calculated with the similarity percentages procedure (SIMPER; Clarke 1993). SIMPER determines which species contribute the most to the similarity and dissimilarity of groups of samples (in our case, years). At the sound station, six of the same nine species (minus winter flounder, scup, and American lobster) had a correlation of 0.96 with the similarity matrix for all 25 species (Fig. 5). These six species accounted for 57% of the dissimilarity between the two clusters of years in Fig. 3*b*.

Total abundance of the 25 species increased over time at both stations (Fig. 6*a*). Abundance peaked between 1993 and 1995 and declined during the past 10 years. The species diversity indices all increased somewhat over time, but for

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Fig. 3. Ordination of the abundances of 25 species in 47 years: (*a*) bay station (Fox Island); (*b*) sound station (Whale Rock). The nonmetric multidimensional scaling (MDS) was based on the Bray–Curtis similarity of square-root-transformed data. Each point represents one year; points that are closer together have more similar species composition than distant points. Symbols indicate the decades: inverted triangles,1960s; squares, 1970s; diamonds,1980s; circles, 1990s; upright triangles, 2000s. The contours enclose clusters with ≥60% similarity. 2D, two dimensional.

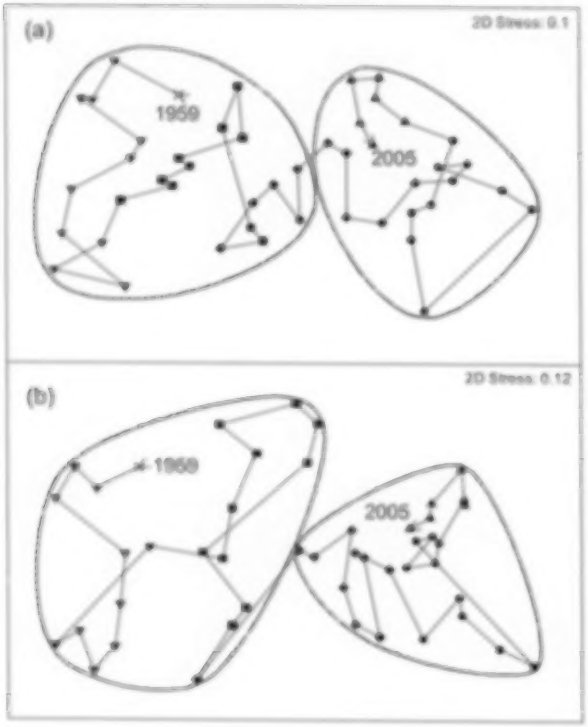
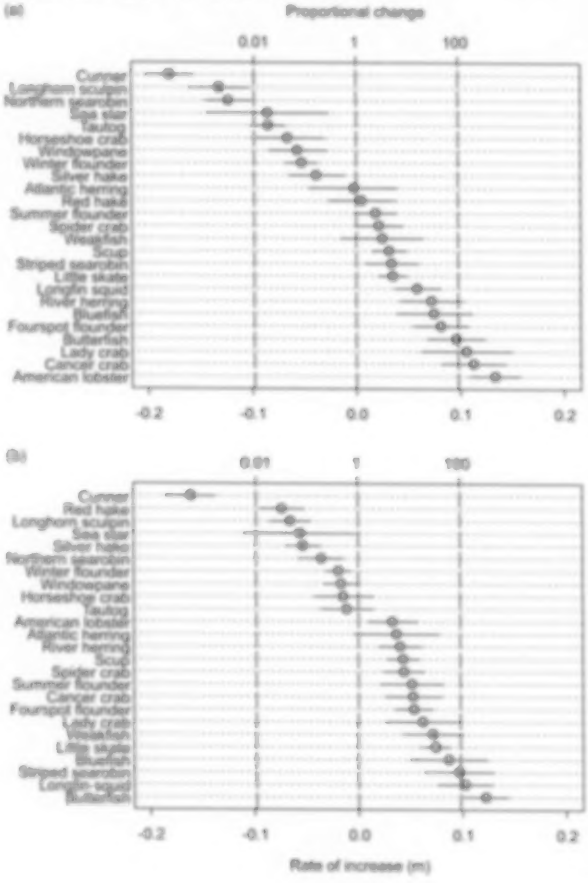


Fig. 4. Rates of increase or decrease of 25 species: (*a*) bay station; (*b*) sound station. The open circles represent the slopes (*m*) from regression of log-transformed abundance against time; the horizontal solid lines are the 95% confidence intervals of the slopes. The broken vertical lines indicate a 100-fold decrease (0.01), no change (1), and 100-fold increase (100) over 47 years.



most of the standard indices, the temporal trends were insignificant. Temporal patterns were much more apparent in diversity indices that incorporate the taxonomic relatedness of the species. Average taxonomic distinctness was lowest in the 1960s when the community was dominated by demersal fish species, which are related at the class and order levels (Fig. 6*b*). Taxonomic distinctness increased significantly with time ($p < 0.001$) at both stations as species from different phyla and classes became abundant, especially Crustacea and Cephalopoda.

In addition to the increase in invertebrates, there was a shift to pelagic species in the 1980s (Fig. 6*c*). Even though the bottom trawl primarily targets demersal species, by 1994, the pelagic–demersal ratio exceeded 1 at both stations and has remained above 0.1 since then. Although mean numbers per tow increased at both stations, the species that became more abundant tended to be ones with smaller body size (Fig. 6*d*). A decline in weighted mean L_{max} is apparent at both stations and was especially pronounced in the sound where mean L_{max} declined by about one-third or 20 cm. Over time, the community shifted toward species with higher temperature preferences (Figs. 6*e*, 6*f*). Mean T_{pref} increased by 2 °C over the 47-year time series according to analysis of covariance (p(equal slopes) = 0.43, p(zero slope) < 0.001). At the bay station, T_{pref} increased with a sinusoidal pattern that was driven by the alternating abundances of winter flounder (cool) and scup (warm) (Fig. 6*e*); the increase in T_{pref} was more linear at the sound station (Fig. 6*f*).

The environmental time series that correlated most strongly with the species dissimilarity matrix were SST and chlorophyll *a* concentration (Table 2). High correlations were found with annual mean SST at all three lags. The highest correlations with temperature were for spring–summer SST with a 2-year lag. Many of the species recruit to the trawl survey at ages 1 and 2; therefore, the lagged SST indices correspond to conditions experienced during spawning and the early life stages. Sea surface temperature had an increasing trend during this period (Fig. 7*a*). Spring–summer SST increased 1.61 °C over the 47-year time series according to analysis of covariance (p(equal slopes) = 0.89,

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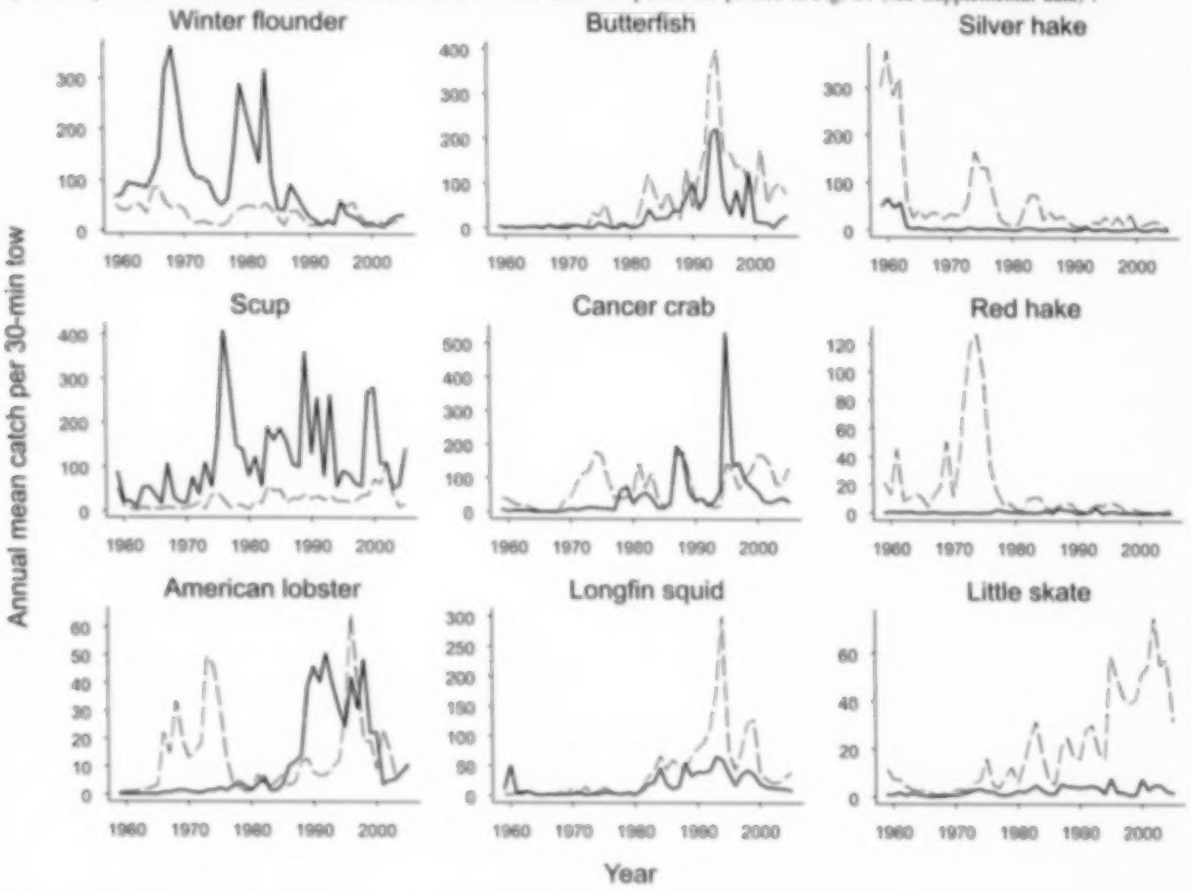
Fig. 5. Annual mean abundances of the nine species that are primarily responsible for the ordinations in Fig. 3: bay station, solid line; sound station, broken line. These nine species account for most of the pattern at the bay station. The six species in the middle and right columns explain the pattern seen at the sound station. Abundances of the other 16 species are plotted in Fig. S1 (see Supplemental data)[2].



p(zero slope) < 0.001). Annual mean SST increased by 1.3 °C over the same time period.

Community composition, as measured by the species dissimilarity matrix, was significantly correlated with chlorophyll *a* concentration (Table 2). The correlation was higher at the bay station than at the sound station, possibly because chlorophyll was measured at a midbay station close to Fox Island. Chlorophyll had a declining trend during this period (Fig. 7*b*). Correlations with chlorophyll were not calculated for lags other than 0 because of the limited number of years and the data gap within the time series. Low and insignificant correlations were found between the species dissimilarity matrix and the number of days fished (Table 2). Fishing effort varied during this time period (Fig. 7*b*), but the pattern did not match the species abundance trends.

The species dissimilarity matrix had intermediate level, but nominally significant correlations with winter NAO at all lags (Table 2). Winter NAO had a generally increasing trend since the 1960s (Fig. 7*c*), which matches some of the trends in species abundance. Winter NAO is correlated with SST, especially winter SST (bay $\rho = 0.56$; sound $\rho = 0.50$), which may explain why both are correlated with the fish species composition. Low and insignificant correlations were found between the species dissimilarity matrix and the Atlantic Multidecadal Oscillation (AMO). The decadal pattern of the AMO (Fig. 7*c*) is quasi-cyclic and does not match the temporal shift in species composition.

We also tested for correlations between community composition and sets of environmental variables (in analogy with multiple regression). However, the differing lengths of the environmental time series limited the combinations of variables we could test (Table 2). For indices with the same number of years, the rank correlations with several variables (e.g., SST, NAO, AMO) were no higher than those for a single variable (SST alone). Therefore we report only the correlation coefficients for single environmental variables in Table 2.

Univariate regressions were performed to identify how individual components of the fish and invertebrate community responded to the environmental factors (Table 3). All of the biotic variables except taxonomic distinctness and mean L_{max} were log-transformed for the linear regressions. Therefore, the magnitudes of the slopes in each column indicate the relative importance of that factor on each biotic variable.

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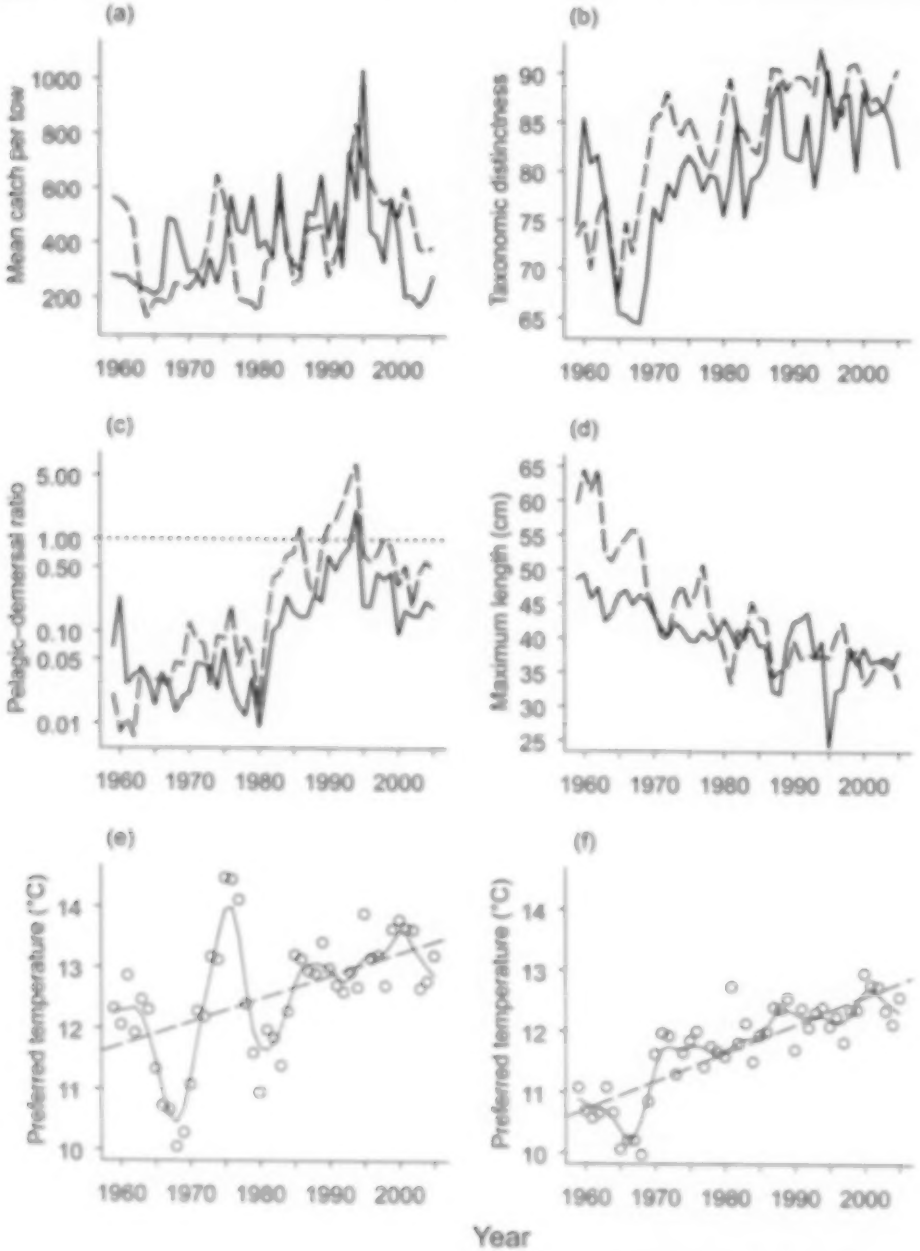
Fig. 6. Community metrics calculated for the bay station (solid lines) and sound station (broken lines): (*a*) total mean catch per 30-min tow; (*b*) taxonomic distinctness (Δ^+); (*c*) pelagic–demersal ratio; (*d*) weighted mean maximum length, L_{max}; (*e* and *f*) weighted mean preferred temperature, T_{pref}, at (*e*) the bay station and (*f*) the sound station. The solid lines in (*e*) and (*f*) are lowess smoothers and the broken lines are the linear time trends.



Squid abundance at both stations was positively related to annual SST and winter NAO and negatively correlated with chlorophyll concentration (Table 3). At the bay station, squid abundance was also positively related to the AMO. The abundance of pelagic fish was also positively related to SST and winter NAO at both stations. At the sound station, pelagic fish were also positively related to fishing effort and negatively related to chlorophyll concentration. Benthic invertebrate abundance was strongly related to annual SST, especially at the bay station. Demersal fish abundance at the bay station was negatively related to the AMO and positively related to chlorophyll concentration; at the sound station, there were no significant regressions for demersal fish.

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Table 2. Rank correlations between environmental variables and fish species composition.

Lag (year)	Annual SST (°C)	Spring–summer SST (°C)	Chlorophyll *a* concentration ($mg \cdot m^{-3}$)	Days fished	Winter NAO	AMO
Bay Station (Fox Island)						
0	**0.26**	**0.27**	**0.40**	0.03	**0.19**	0.02
1	**0.23**	**0.38**	NA	0.04	**0.18**	0.01
2	**0.27**	**0.39**	NA	0.07	**0.17**	0.03
Sound Station (Whale Rock)						
0	**0.29**	**0.28**	**0.33**	0.03	**0.16**	0.05
1	**0.28**	**0.33**	NA	0.07	**0.18**	0.05
2	**0.30**	**0.37**	NA	0.09	**0.15**	0.02
No. of years	47	47	25	42	47	47

Note: The BIOENV procedure was used to calculate the rank correlation between the distance matrix of the environmental variables and the dissimilarity matrix of abundance data. Significance levels were determined with 999 replicates of a randomization test; bold type indicates $p < 1\%$. SST, sea surface temperature; NAO, North Atlantic Oscillation; AMO, Atlantic Multidecadal Oscillation; NA, not available.

Taxonomic distinctness increased with SST (Table 3). It was also positively related to the AMO at the bay station and with winter NAO at the sound station. The pelagic–demersal ratio (*P:D*) increased with SST, winter NAO, and the number of days fished. At the sound station, the *P:D* was also positively related to chlorophyll concentration. There was a strong negative relationship between the mean L_{max} and annual SST. At the bay station, there was also a negative relationship between mean size and days fished. In summary, the signs of the regression coefficients were consistent between the two stations. There were consistent effects of SST on all of the biotic variables except demersal fish. Winter NAO was consistently related to the pelagic component of the community. Chlorophyll concentration was generally negatively related to the pelagic component of the community except the *P:D* at the sound station.

Discussion

The species composition of the fish and invertebrate community in Narragansett Bay and adjacent Rhode Island Sound shifted dramatically between 1959 and 2005. A gradual transition from fish to invertebrate species was punctuated by an abrupt shift from a benthic to a pelagic regime between 1980 and 1981. Given the abruptness of this transition, it could be considered a regime shift (Collie et al. 2004). Although efforts have been made to standardize the operation of the fish trawl, the timing of species shifts could be confounded with necessary changes in fishing boats over the years. Species-specific catchability could alter in response to changing temperature or predator abundance. We believe that any potential vessel or catchability effects would be minor compared with large shifts in species composition observed. Our results are consistent with those of the Rhode Island Department of Environmental Management trawl survey, which started in 1979 and overlapped half of our time series. Though data are missing for the first two decades, demersal fish species have become less numerous, relative to pelagic species, since 1979 (Oviatt 2004). We conclude that the shift in species composition is real and not a sampling artifact.

Several of the community metrics calculated in this study capture the major shifts in species composition. Taxonomic distinctness increased at both sites as the dominant benthic fish were replaced with a mixture of fish and invertebrate species. The *P:D* is a sensitive indicator that has been related to nutrient enrichment of inland seas (de Leiva Moreno et al. 2000). However, increases in *P:D* have also been observed in continental-shelf communities (Fogarty and Murawski 1998; Choi et al. 2004) where anthropogenic eutrophication has not occurred. If fisheries target demersal species, the *P:D* is expected to increase with fishing (Rochet and Trenkel 2003).

The mean L_{max} index was also very sensitive to the shift in community structure. It can be a useful proxy for the actual mean size in the community for situations in which not every individual is measured. The decline in mean size is consistent with the prediction that fishing will shift the size composition of a community toward small species with shorter life spans (Rochet and Trenkel 2003). Fishing also truncates the age structure, reducing mean size within species and, in extreme cases, selecting for genotypes with smaller size at age (Law 2000). To the extent that fishing caused the shifts in Narragansett Bay, the observed declines in mean L_{max} are a conservative measure of the decline in mean size. A shift toward smaller body size is also predicted as a physiological response to global warming. The increase in mean T_{pref} paralleled the increase in SST, supporting the hypothesis that southern species are replacing northern ones.

In summary, these community metrics capture the dominant patterns in the multivariate data, particularly the long-term shifts. These metrics are very useful for making comparisons with other communities, which may have similar dynamics but different species identities. For example, de Leiva Moreno et al. (2000) compared the *P:D* among 14 semi-enclosed seas around Europe. Geographic comparisons of community metrics may help to elucidate the cause of long-term shifts in species composition. Temporal changes in the community metrics are consistent with several hypotheses, but none of them can be considered exclusive. Likewise, there is no reference or baseline level for an unimpacted community. Notwithstanding the long time series and the fact that the most rapid changes in Narragan-

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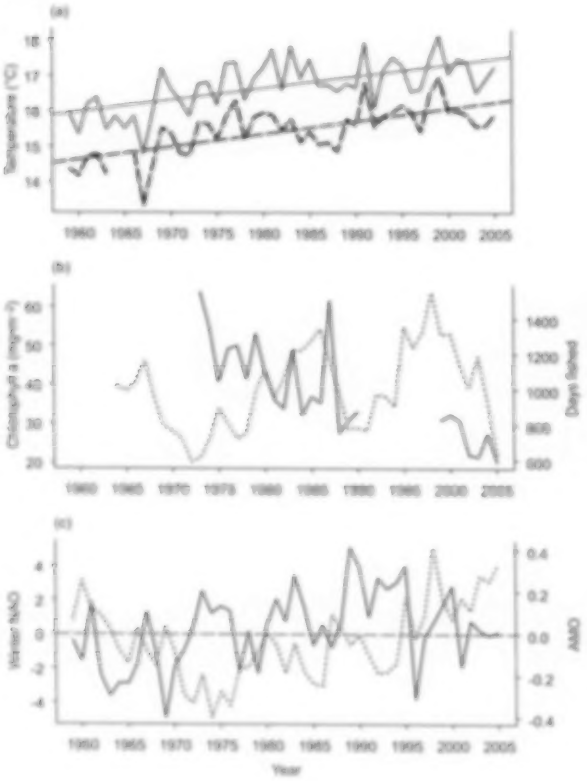
Fig. 7. Environmental indices. (*a*) Average spring–summer sea surface temperature and corresponding linear regression lines (bay station, solid lines; sound station, broken lines). (*b*) Annual mean chlorophyll *a* concentration (solid line). The 1973–1990 chlorophyll data are from Li and Smayda (1998); the 1999–2005 data are from http://www.gso.uri.edu/phytoplankton. Nominal trawl fishing effort in Northeast Fisheries Science Center (NEFSC) statistical area 539, which includes Rhode Island Sound, at the mouth of Narragansett Bay (dotted line). Days fished were normalized by Marc Terceiro (NEFSC, 166 Water Street, Woods Hole, MA 02543, USA, personal communication, 2006) as hours fished/24. (*c*) Winter North Atlantic Oscillation index (NAO, solid line) from http://www.cgd.ucar.edu/cas/jhurrell/indices.html. Atlantic Multidecadal Oscillation (AMO, dotted line) from http://www.cdc.noaa.gov/Timeseries/AMO.



sett Bay have probably occurred in the period since 1960, the Bay has been continuously fished for over 100 years and numerous changes to the habitat and fauna occurred during that time (Oviatt et al. 2003). Therefore, the species that were present in the 1960s may already have constituted a "shifted baseline."

Mounting evidence has revealed that even small increases in water temperature over extended periods of time can directly influence the species composition, distribution, and abundances of surrounding fish communities (Murawski 1993; Genner et al. 2004; Perry et al. 2005). Sea surface temperature in the English Channel and Bristol Channel, UK, increased by over 1 °C from 1905 to 2005 (Genner et al. 2004). Compositional changes resulting from SST warming were observed in both locations; various opportunistic species, already present in the community, flourished in the warmer conditions and quickly became the dominant species. Temperature-driven changes in species distributions have been observed elsewhere in the northwest Atlantic. Variations in water temperature were significant in explaining changes in mean latitude of occurrence of 12 of 36 species (Murawski 1993). Weighted mean catches of these species shifted poleward by 0.5°–0.8° latitude (55–89 km) for each 1 °C increase in water temperature. Even larger distributional shifts have been observed in the North Sea in response to a 1 °C increase in sea temperature from 1977 to 2001 (Perry et al. 2005). The average shift for 15 fish species was 1.6° latitude (172 km); 13 of these 15 shifts were poleward.

A similar mechanism may be acting in Narragansett Bay, with temperature increases influencing species distributions and resident species being replaced by opportunistic seasonal migrants. In situ SST data had the highest and most consistent correlations with species composition and abundance in both the univariate and multivariate tests. Temperature increases correlated with the increased abundance of squids, pelagic fish, benthic invertebrates, taxonomic distinctness, and the *P:D*; mean L_{max} was negatively correlated with SST. The 1.6 °C increase in winter temperature is correlated with the decline of various species that reside in Narragansett Bay during the cold winter months (e.g., winter flounder, silver hake, and red hake). These sub-boreal species have been replaced by seasonal migrants that are more abundant during the summer months (e.g., butterfish and scup). Estuarine species with discrete subpopulations (e.g., winter flounder, tautog, cunner) may not be able to shift their distribution rapidly as temperature increases, and their decline may therefore be an early indicator of global warming.

In addition to in situ SST, there were weaker, but still significant, correlations with basin-scale climate indices. Winter NAO was positively correlated with the pelagic component of the community and the *P:D*. These correlations suggest that the shift from a benthic- to pelagic-dominated community may have been triggered by the winter NAO, which has switched to predominantly positive values since 1980 (Hurrell 1995). The NAO has previously been correlated with the abundance, growth, and migration phenology of estuarine fish (Attrill and Power 2002; Sims et al. 2004). Finally, the increase in the AMO was correlated with the decline in demersal fish at the bay station. The basin-scale indices may reflect environmental conditions in the broader region occupied by migratory species.

There were no strong correlations between species composition and fishing effort, which varied by a factor of two since 1964. The number of days fished was positively correlated with the pelagic component of the community and negatively correlated with L_{max} at the bay station, as would be predicted from fishing effects. Admittedly, a single index of fishing effort does not permit a strong test of fishing effects. For fished species, one would expect a negative relationship between abundance and fishing effort, but that this relationship would occur with time dynamics and lags. Fishing is implicated in the decline of some of the commercially important fish species (e.g., winter flounder, tautog).

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Table 3. Linear regressions of biotic variables against potential explanatory variables.

	Annual SST		Winter NAO		Annual AMO		Days fished		Chlorophyll *a*	
Variables	Slope	*p*	Slope	*p*	Slope	*p*	Slope	*p*	Slope	*p*
Bay Station (Fox Island)										
Squid	0.783	0.007	0.207	0.006	0.807	0.020			–0.044	0.006
Pelagic fish	0.921	0.004	0.246	0.004						
Benthic invertebrates	1.264	<0.001								
Demersal fish					–1.053	0.005			0.021	0.005
Taxonomic distinctness	3.507	0.032			10.355	0.030				
Pelagic–demersal ratio	0.693	0.026	0.202	0.013			0.002	0.043		
Mean L_{max} (total length)	–3.142	0.008					–0.008	0.009		
Sound Station (Whale Rock)										
Squid	1.028	0.014	0.324	0.004					–0.062	0.007
Pelagic fish	1.322	<0.001	0.246	0.007			0.002	0.030	–0.048	0.012
Benthic invertebrates	0.662	0.002								
Demersal fish										
Taxonomic distinctness	5.678	<0.001	0.759	0.033						
Pelagic–demersal ratio	1.236	0.002	0.247	0.015			0.002	0.048	0.002	0.034
Mean L_{max} (total length)	–9.044	<0.001								

Note: Only slopes with associated probability ($p < 0.05$) are reported. SST, sea surface temperature; NAO, North Atlantic Oscillation; AMO, Atlantic Multidecadal Oscillation.

However, fishing mortality rates are not available for all of the dominant species, which makes it difficult to quantify the impacts of fishing at the community level. Some unfished species (e.g., cunner, northern searobin) have declined dramatically, whereas some of the species that have increased are now the targets of commercial fisheries (e.g., butterfish, scup, longfin squid). Although fishing may have partly triggered the shift, the fisheries also responded opportunistically to the more recent species composition.

Some of the changes in species composition can be interpreted as releases from competition or predation. The decline of several fish species that feed primarily on crustaceans (red hake, northern searobin, longhorn sculpin) and corresponding increase in *Cancer* crab and American lobster is consistent with a release from predation (Worm and Myers 2003). The increase in little skate abundance at the sound station mirrors coastwide increases in skate abundance during the same period and may be attributed to less competition or predation release (Myers et al. 2007). The reduction in silver hake, a dominant piscivore, may have contributed to the increase in forage fish species (butterfish, longfin squid). However, other piscivore species (e.g., bluefish and striped bass) increased during the same period.

A significant correlation was found between the fish species composition and chlorophyll *a* concentration in Narragansett Bay. The decline in chlorophyll *a* was correlated with increases in squid and pelagic fish and declines in demersal fish. The two-thirds decline in chlorophyll *a* is surprising given that nutrient inputs to Narragansett Bay have changed little during this period (Nixon et al. 2007). One explanation for the reduction in chlorophyll is a reduction in the magnitude of the spring bloom in response to increased water temperatures (Oviatt 2004). According to this explanation, warmer temperatures have increased zooplankton grazing rates such that more of the primary productivity is grazed in the water column, with less accumulating and sinking to the benthos. The coincident decrease in chlorophyll *a* and increase in the *P*:*D* in Narragansett Bay is opposite to the positive relationship between log(*P*:*D*) and log(Chl *a*) observed among European semi-enclosed seas (de Leiva Moreno et al. 2000).

A shift from benthic to pelagic consumers is also evident in the feeding habits of the 25 top species. The community has shifted from fish that specialize on molluscs and crustaceans to unspecialized predators of benthic and pelagic invertebrates. Several of the benthic species that were more abundant in the 1960s (tautog, cunner, sea stars) feed primarily on mollusks, which presumably were also more abundant then. This dietary shift is consistent with a shift in production from benthic filter feeders (e.g., bivalve molluscs) to enhanced pelagic production (e.g., ctenophores). A shift in production has not yet been documented in Narragansett Bay, but it is consistent with similar benthic-to-pelagic shifts in other estuaries, especially Chesapeake Bay (Jackson et al. 2001).

In summary, there are two long-term patterns in the GSO trawl survey data. The gradual decline of demersal fish, increase in invertebrates, and decrease in mean size in the community appear to be driven primarily by increased SST and secondarily by fishing. We attribute the more rapid shift from benthic to pelagic species starting in 1980 to changes in the pelagic food web that strongly correlate with an increase in winter NAO and a reduction in chlorophyll *a* concentration. Are the observed shifts in species composition reversible? Does the more recent community structure represent an alternate stable state, or will the community structure continue to shift? Pelagic species have become less dominant since 1994, but there is little indication that the species composition is returning to that of the 1960s. If the species composition is largely driven by environmental forcing (SST, NAO) and coastal temperatures continue to increase, it is likely that the fish community will continue

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to shift toward a more warm-water and pelagic community resembling those of more southern estuaries such as Delaware Bay and Chesapeake Bay.

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Estimating walleye (*Sander vitreus*) density, gear catchability, and mortality using three fishery-independent data sets for Oneida Lake, New York

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Abstract: We used three long-term data sets (gill nets, trawls, and adult population estimates) for walleye (*Sander vitreus*) to simultaneously estimate density, gear catchabilities, and mortality using an age-structured, nonlinear model. Model constraints included a fixed natural mortality rate and age- and gear-specific but time-invariant catchabilities. Trawl catchability decreased with age, whereas gillnet catchability increased towards a maximum by age-4. A sensitivity analysis was conducted to investigate how the information content of the different data sets influenced parameter estimates. Estimated catchability values were relatively robust to changes in data weighting. Estimated gillnet catchability values were fairly consistent with those derived from more conventional methods. An additional mortality term was added to reflect double-crested cormorant (*Phalacrocorax auritus*) predation, and vulnerabilities associated with angling and cormorants were calculated using independent length frequency information. Estimated subadult mortality increased and the influence of fishing mortality slightly decreased during recent years when double-crested cormorants were abundant and more restrictive size limits were in place. Walleye density in Oneida Lake (New York, USA) in the last decade is estimated to be approximately half of that before 1990.

Résumé : Nous utilisons trois banques de données à long terme (récoltes au filet maillant et au chalut, estimations de la population adulte) pour déterminer simultanément, à l'aide d'un modèle non linéaire structuré en fonction de l'âge, la densité, la capturabilité aux divers engins et la mortalité du doré (*Sander vitreus*). Les contraintes du modèle incluent un taux de mortalité naturelle fixe et des capturabilités spécifiques à l'âge et à l'engin, mais invariables dans le temps. La capturabilité au chalut diminue avec l'âge, alors que la capturabilité au filet maillant augmente pour atteindre un maximum à l'âge de quatre ans. Une analyse de sensibilité a servi à vérifier comment le contenu informatif des banques de données influence l'estimation des paramètres. Les valeurs estimées de capturabilité sont relativement robustes au changements de pondération des données. Les valeurs de capturabilité au filet maillant s'accordent généralement avec celles obtenues par des méthodes plus conventionnelles. Nous avons ajouté un terme de mortalité pour tenir compte de la prédation par les cormorans à aigrettes (*Phalacrocorax auritus*); des vulnérabilités associées à la pêche sportive et aux cormorans ont été calculées à l'aide d'information indépendante sur les fréquences de longueurs. La mortalité estimée des subadultes a augmenté et l'influence de la mortalité due à la pêche a diminué au cours des dernières années, alors que les cormorans à aigrettes étaient abondants et que des limites de taille plus restrictives ont été appliquées à la pêche. Nous estimons que la densité des dorés dans le lac Oneida (New York, É.-U.) durant la dernière décennie était d'environ la moitié de celle d'avant 1990.

[Traduit par la Rédaction]

Introduction

A common objective of traditional fisheries catch-at-age models is to use observed catch statistics to estimate both population abundance and exploitation rates through time (Hilborn and Walters 1992). Virtual population analysis (or cohort analysis) uses backwards projections to estimate previous abundance levels, whereas integrated analysis (or statistical catch-at-age analysis) estimates abundances for all ages during the initial year and for the age at first recruitment for all subsequent years to then project the population forward through time (Haddon 2001). A key component of

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these models is catch-at-age data from the fishery, which reflects the combination of catchability and population abundance at age. In addition, combining data from several sources (e.g., fishery-dependent and fishery-independent indices) can improve stock assessments (Maunder 2001). These methods are often applied to commercial fisheries data where catch-at-age information is abundant. In the case of some sport fisheries, managers may not have fishery catch data available but may still have several independent indices of fish abundance. In these cases, multiple gear types often have been used to sample a fish population because gears typically vary in their ability to capture fish of different ages. Consequently, the resulting data provide different information, often producing a more complete picture of the fish population than any single data source on its own (Olin and Malinen 2003). However, multiple measures can be difficult to reconcile into a best estimate of the development of a specific population over time because differences in gear selectivity result in different catchabilities across age or size groups (e.g., Hamley 1975; Fernö and Olsen 1994; Jackson and Noble 1995). As a result, each data series is often treated as a separate index of abundance. A better use of independent data sets may be to combine them into a single maximum likelihood estimate of the fish population of interest.

Here, we combine three long-term data series for walleye (*Sander vitreus*) from Oneida Lake, New York, to examine the nature of the dynamics of this species and to use multiple sources of information to assess important population parameters. There are no commercial fisheries in Oneida Lake and only periodic estimates of recreational angler harvest exist (VanDeValk et al. 2005*a*), but standardized, fishery-independent sampling has produced three long-term data sets, each of which provides a measure of walleye abundance at multiple ages. The absolute abundance of adult (age-4 and older) walleye has been estimated by mark–recapture for 21 nonconsecutive years since 1958. In addition, walleye in Oneida Lake have been routinely collected in gill nets and trawls with known effort for nearly half a century, and individual fish have been subsequently aged. While standardized gillnet and trawl collections provide the opportunity for independent, age-specific estimates of relative abundance over time, both gears are size selective.

For both of our sampling gears, we relate catch to density estimates using gear-specific catchability-at-age values. Catchability is typically defined as the proportion of total population abundance that is captured per unit effort and generally is a value much less than 1. However, if the population is defined as a density (abundance per unit area), there is no theoretical upper limit to catchability, and values may be substantially higher than 1. Gear selectivity is usually presented relative to a category (e.g., age or size), with one or more categories characterized as being fully selected (e.g., a fully selected age). In this paper, we do not distinguish between these terms and discuss gear-specific catchability-at-age values (e.g., a combination of fully-selected catchability and age-specific gear selectivity; Quinn and Deriso 1999) as age-specific scalars that relate the density of an age group to the age-specific catch for a given gear (see Materials and methods for more specific details on estimated parameters).

The ability of a sampling gear to capture organisms can depend on several factors. Anderson (1998) describes encounter, contact, and retention components of multifilament gill nets. These components are influenced by the size of the fish relative to that of the mesh, the visibility of the net, as well as the swimming speed and activity level of a fish. For example, swimming speed tends to increase with fish size, leading to increased encounter probability. Likewise, larger mesh sizes have a less visible surface, which increases contact probability, while larger fish can also be entangled in small mesh sizes. Combined, these effects suggest that gillnet catchability should increase with fish size even if the maximum retention probabilities of various mesh sizes are the same (Hamley 1975; Rudstam et al. 1984; Grant et al. 2004). In contrast, trawl catchability likely decreases with fish size because increased swimming speed at larger sizes should improve the ability of fish to avoid the gear (Wardle 1983). In addition, trawl catchability can be affected by water clarity (Nielsen 1983; Buijse et al. 1992), towing speed, and trawl material and construction (Wardle 1983). Variation in behavior, activity periods, and habitat preference among life stages of fish can further bias catches in most gears (Willis et al. 1985; Rudstam and Johnson 1992). In the case of these and other sampling approaches, gear biases and sampling variability can cause catch data to misrepresent the abundance of different age groups or size classes. As such, it is necessary to account for these differences in gear catchability to effectively compare age-specific estimates across sampling techniques.

We developed an estimation model that simultaneously uses all available long-term data for walleye in Oneida Lake to obtain a maximum likelihood estimate of the population while following multiple age groups over time. Specifically, our objectives were to synthesize the three fishery-independent, long-term data sources for Oneida Lake walleye to (*i*) derive an internally consistent and age-specific assessment of walleye density across nearly 50 years of data collection, (*ii*) estimate age-specific catchability values for both an active and a passive sampling gear, and (*iii*) evaluate possible changes in walleye mortality associated with an increase in abundance of a predator — the double-crested cormorant *Phalacrocorax auritus*.

Based on the available data and estimated parameters, it was not possible to allow the model to estimate a separate mortality rate for each year. However, we do expect a change in walleye mortality over time based on changing conditions in Oneida Lake. During the early 1990s, the establishment of zebra mussels (*Dreissena polymorpha*) (possibly combined with decreased nutrient loading) led to increased water clarity and photic zone depth in Oneida Lake (Idrisi et al. 2001), causing shifts in lower level trophic dynamics and enhanced littoral macrophyte growth (Mayer et al. 2002; Zhu et al. 2006). At the same time, the abundance of double-crested cormorants on Oneida Lake has increased, introducing a new source of predation on subadult walleye (VanDeValk et al. 2002; Rudstam et al. 2004; Coleman et al. 2005). Rudstam et al. (2004) documented increased mortality of ages 1–3 walleye in the 1990s (based on trawl catches) and argued that cormorants were a major cause of the decline in the walleye population in Oneida Lake during this time. In addition, size limits for anglers

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have changed over time in the lake, likely contributing to differences in fishing mortality. Therefore, we decomposed mortality into three components: natural mortality, cormorant mortality, and fishing mortality. An instantaneous natural mortality was calculated from independent data (see Materials and methods) and fixed over all ages and years, whereas cormorant and fishing mortality were estimated and their influence varied with the proportion of vulnerable fish present in an age group. For fishing mortality, this proportion is a function of the angling regulation (size limit) imposed in specific years. The vulnerability of various ages to cormorant mortality also was based on fish size but only occurred during years after 1990, the year Rudstam et al. (2004) suggested cormorant predation had become noteworthy In addition to creating a more realistic, time-dependent mortality, this allowed us to further test the conclusion of Rudstam et al. (2004) that subadult mortality in Oneida Lake walleye had increased since 1990.

We used a nontraditional, age-structured, nonlinear population dynamics model to estimate age-specific gear catchabilities, mortality rates, and the density (number·ha^{-1}) of multiple age classes of walleye using input data from all three long-term data sets for walleye in Oneida Lake. The sensitivity of the catchability estimates to the selected weighting of the three data sources or to the fixed natural mortality rate was then investigated. We also compared gear catchability-at-age estimates from the model with both direct measures from the adult population and a gillnet relative selectivity curve constructed with traditional methods using the length distribution of the catch in individual mesh sizes.

Materials and methods

Description of study site

Oneida Lake has the largest (207 km^2) surface area of any lake entirely in New York State and is one of the most studied freshwater ecosystems in North America (Adams and Hankinson 1928; Mills et al. 1978; Forney 1980). In addition to offering long-term data series on multiple trophic levels ranging from algae to top predators, Oneida Lake provides important recreational and economic resources to much of central New York and the northeastern USA. Walleye and yellow perch (*Perca flavescens*) are the most sought after sport fishes, and both species are important components of the Oneida Lake food web (Forney 1980; Mills and Forney 1988). Nearly every spring, larval walleye are stocked into Oneida Lake soon after hatching, and most captured walleye fry are believed to be of hatchery origin (Houde and Forney 1970; Forney 1975, 1980).

Indices of walleye abundance

Gillnet catch data for walleye are available for all years from 1958 to 2003 except for 1974. In each year, multifilament gill nets were used to sample 15 fixed sites in a standardized sequence from June through September. At each site, two nets (300 ft × 6 ft depth; 1 ft = 0.3048 m) were set on bottom and allowed to fish overnight for approximately 12 h. Each net consisted of two gangs of six 25-ft panels with mesh size (as stretch mesh) that ranged from 1.5 inches to 4 inches at 0.5-inch increments (1 in = 25.4 mm). From 1963 through 1967, gillnet sampling was conducted using one white net and one colored (red, green, or black) net. During other years, two white nets were used at each site. Each year, scales were removed from all or a subsample of captured walleye for age determination, and these data were combined across sites and used to determine the annual age distribution for the total catch. The gillnet catch data were combined across mesh sizes and sampling locations so that annual total catch-at-age values could be used as an input data set for the estimation model.

Walleye abundance in Oneida Lake has also been assessed with a summer–fall bottom trawl survey at 10 standardized sites since 1961. Each site was sampled weekly for about 15 consecutive weeks. The bottom trawl had a 5.5 m footrope, and each haul sampled approximately 0.1 ha of bottom surface area with a transect length of approximately 280 m (see Nielsen 1983 for more details). Trawl catch data were combined across sites within a year. To facilitate comparisons with population estimates, we converted total trawl catches to catch per hectare swept (henceforth referred to as trawl CPUE). As for gill nets, trawl CPUE-at-age was used as an input data set. During 7 years, age-7 walleye were not specifically identified in the ageing process for trawl-caught fish. Instead, they were included as part of an age-7 and older group. These few lumped-age estimates were not used as trawl input data for the estimation model (see below).

Since 1958, the population size of adult walleye has been periodically estimated using mark–recapture methods (N = 21 years). Unlike the long-term data sets from trawl and gillnet sampling, the mark–recapture population estimates are for adult fish only. During early spring (usually April), adult walleye were collected in trap nets near Scriba Creek by the Oneida Fish Cultural Station staff, Constantia, New York, and near Shackelton Point by personnel from the Cornell Biological Field Station. Individual walleye were marked (12 000 – 26 000 per year) using a year-specific fin clip. Occasionally, additional walleye were captured and marked at other locations using trap nets and electrofishing surveys; however, these collections usually contributed less than 20% of the total number of marked fish. Throughout the summer and fall, recaptures were collected primarily using electrofishing and large trawls (with approximately equal effort in all areas of the lake), but occasionally using gill nets and trap nets (during 1958–1960). For the years when mark–recapture methods were employed, population estimates were calculated for each age group of adult walleye using Bailey's (1951) modification of the Petersen formula (additional details for Oneida Lake in VanDeValk et al. 2002) and used as an input data set for the estimation model.

Parameter estimation

We used AD Model Builder software (Otter Research Ltd., Sydney, British Columbia) to construct and perform nonlinear estimation procedures. Based on the relative availability of the three input data sources, the estimation model followed age-1 through age-7 and an aggregate age-8 and older category of walleye during the period 1958 through 2003. We used the model to evaluate age-specific catchability values for both sampling gears. The few missing trawl values and the missing gillnet catch from 1974 were not

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Table 1. Adult (age-4 and older) walleye (*Sander vitreus*) survival, abundance, and angler exploitation data used to select initialization values for mortality parameters.

Year	Annual survival	Adult abundance	Angler harvest	Z	F	M
1958	0.87	1 035 000	106 000	0.139	0.110	0.030
1959	0.46	960 800	449 000	0.777	0.672	0.104
1997	0.80	251 900	35 400	0.223	0.157	0.066
2002	0.79	345 900	9 587	0.236	0.031	0.205
Average				0.344	0.242	0.101

Note: Instantaneous total mortality rates (Z) were partitioned to instantaneous fishing (F) and instantaneous natural mortality (M) rates by setting the ratio of F to M equal to the ratio of number harvested by angling to number lost to natural mortality using independent estimates of walleye survival and angler harvest.

used in model estimation; their contribution to the likelihood was eliminated using a matrix of weighting factors (0 or 1). Age-0 data were not included in the estimation model because of the very low catchability of this age class in gill nets as well as known high variability in annual mortality due to the vulnerability of this age class to cannibalism (Chevalier 1973). Estimated parameters included age-specific catchability values for the trawl and gill net separately, an instantaneous fishing mortality rate, an instantaneous mortality rate reflecting double-crested cormorant predation, and an initialization vector (including age-1 recruitment) of walleye density (number·ha^{-1}). Parameters were initialized and estimated as $\log_e$ values.

We used available walleye survival and harvest data to calculate a fixed instantaneous natural mortality rate and an initialization value for instantaneous fishing mortality. Annual measures of adult walleye survival and angler harvest were available during 1958, 1959, 1997, and 2002 (Forney 1967; VanDeValk et al. 2005*b*; Table 1). Annual survival measures (Table 1) were calculated as an average of two independent methods (VanDeValk et al. 2005*b*), one based on decreased population size over time and the other based on the ratio of different year-specific marks (see Ricker 1975). The corresponding instantaneous total mortality rates were then partitioned to instantaneous fishing (F) and instantaneous natural mortality (M) rates by setting the ratio of F to M equal to the ratio of number harvested by angling to number lost to natural mortality, using estimates of survival and angler harvest for years when these data were available (Table 1). Next, these values were averaged over years (Table 1), and an instantaneous natural mortality rate of 0.101 was applied to all age classes in the estimation model. The calculations used to determine M were based on data for adult walleye, but we felt that it was reasonable to also apply this rate to subadults because throughout most of the sampled period, we expect that late-year age-1 and age-2 walleye would have few predators as they outgrow the size-selective piscivorous fish present (Chevalier 1973), and anglers harvest few walleye at these young ages (VanDeValk et al. 2005*a*). As part of a sensitivity analysis (described below), we also reran the model using either one-half or twice the calculated value of M. Likewise, an F parameter was initialized at 0.242 (but then estimated by the model) based on data for these years (Table 1).

Cohorts were modeled to follow an exponential decline with time:

$$(1) \qquad N_{i+1,j+1} = N_{i,j}\, e^{-Z}$$

where $N_{i,j}$ is the density (number·ha^{-1}) of a cohort during year i at age j, $N_{i+1,j+1}$ is the density of the same cohort during the following year, Z is an instantaneous annual mortality rate (all sources), and t represents a 1-year time step. The density parameters for the initial year (1958) and the age-1 recruitment parameters were estimated on the $\log_e$ scale and, for the efficiency of the algorithm, as an overall mean coupled with bounded deviations. Density was initialized with a value of 33.1 based on the average catch of age-1 walleye in the trawl and a previous trawl-catchability estimate for adult walleye.

For both the gill net and trawl, we assumed catchability was age-specific but remained constant across years. The annual age-specific catches for both gears were estimated from the predicted population density of walleye and the age-specific catchability scalar:

$$(2) \qquad C_{i,j,k} = N_{i,j} q_{j,k}$$

where $C_{i,j,k}$ is the predicted catch at age-j of walleye in the kth sampling gear during year i, $N_{i,j}$ is the estimated density (number·ha^{-1}) of age-j walleye in the lake during year i, and $q_{j,k}$ is the estimated age-specific catchability for that gear. The reported catchability-at-age estimates for the gill net do not range from 0 to 1 because the gillnet data are on the scale of annual total catch-at-age, while the mark–recapture population estimates for adult age classes are density values (number·ha^{-1}). The trawl catchability-at-age values do range from 0 to 1, but only because the area swept by the combined 10 trawls in the standard survey happens to equal 1 ha, the same area as used for density values.

The estimation of parameters was driven by a nonlinear estimation procedure, which arrived at a set of parameter values that minimized a concentrated form of the negative log-likelihood by simultaneously minimizing the differences between the observed and predicted values for gillnet catches, the trawl CPUE, and the adult mark–recapture population estimates (the objective function). We used concentrated forms of the negative log-likelihood functions for normal and lognormal distributions to characterize these re-

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lationships between observations and predictions. For example, the traditional form of the full negative log-likelihood for the normal distribution was reduced to a concentrated form (e.g., $[-\ln L]$) by replacing σ^2 with its maximum likelihood estimate and simplifying:

$$(3) \qquad [-\ln L] = \frac{n}{2}\ln\left(2\pi\frac{\sum_i\sum_j(\text{obs}_{i,j}-\text{pred}_{i,j})^2}{n}\right)+\frac{n}{2}$$

where $\text{obs}_{i,j}$ is the observed measure and $\text{pred}_{i,j}$ is the predicted measure of age-j walleye in Oneida Lake during year i. The mark–recapture data were fit assuming normal error (above), whereas the gillnet and trawl data were fit assuming lognormal error. Lognormal errors were selected for the sampling gear data because of skewness in their residuals when a normal likelihood was used (lognormal likelihood places less influences on extreme values). Because of a few age-specific cases where no fish were caught, we added a minimal value for each gear type (added value was equal to half of the minimum observed value: 0.5 for gill nets and 0.02 for trawl CPUE) to both the observed and predicted values before taking their natural logs. The objective function was the sum of the three separate, concentrated, negative log-likelihood functions, which were specific to each of the input data sets, after weighting coefficients were applied.

Weighting coefficients are usually applied to the different components of the likelihood to characterize the degree to which the separate sets of information should contribute to the overall parameter estimation. In our case, the data for adult walleye density and trawl CPUE were both input into the model as number per hectare; however, the sampling area of the passive gill net was unknown. Differences in the unknown age-specific catchabilities of the passive and active gears also add to the complexity. We applied weighting coefficients to the concentrated log-likelihood functions so that the importance of fitting predicted values to the observed data was approximately equal across the three data sets. To further assess the sensitivity of the catchability estimates to our assumptions, we re-estimated the model parameters under three alternative weightings. In each case, we adjusted the weighting coefficient of the likelihood component of an input data set by a factor of 10, which allowed that data set to dominate the influence of the parameter estimation over the other two. Further, we also examined the sensitivity of the catchability estimates to the assumed fixed natural mortality rate by re-estimating these values using either one-half or twice the calculated value of instantaneous natural mortality.

To evaluate the effects of double-crested cormorant predation on the dynamics of the walleye population, we split the estimation of mortality into two time periods (1958–1989 and 1990–2003). To do this, we added an additional mortality term to the second time period to reflect predation associated with double-crested cormorants. Because age-specific vulnerability to harvesters (either anglers or cormorants) is likely to change over time, we calculated the proportion of each walleye age group that was vulnerable to either anglers or double-crested cormorants using annual total-length-at-age values back-calculated from fish caught in gillnet collections, when the number observed by age category exceeded five, and specified thresholds of vulnerability (e.g., annual angler size limits and a maximum consumable size for cormorants). For the few years when observed catch-at-age was less than five, we calculated an average vulnerability proportion using surrounding years that had the same recreational size limit in effect. This way, the annual vulnerability proportions for angling reflected the effects of that year's minimum length limit for angler harvest from Oneida Lake. Prior to 1975, no minimum size limit was in place, so we assumed that anglers would not have harvested walleye that were less than 12 inches (305 mm). In 1979 and 1980, the minimum length limit was 12 inches, and it was increased to 18 inches (457 mm) from 2001 to 2003. For all other years, the minimum length limit was 15 inches (381 mm). We did not attempt to account for changes in the creel limit. For the age-specific vulnerabilities associated with predation from double-crested cormorants, we similarly used the average length-at-age values and assumed simply that double-crested cormorants were only capable of consuming walleye less than 360 mm (J. Coleman, Cornell University, Department of Natural Resources, Ithaca, NY 14853, USA, personal communication, 2006).

The proportion vulnerable was calculated annually and used to weight the influence of the estimated instantaneous mortality rates across age classes. Using fishing mortality as an example, an age group (N_j) can be separated into vulnerable (V_j) and invulnerable (refuge, R_j) components as

$$(4) \qquad N_j = V_j + R_j$$

Fishing mortality then is only applied to the vulnerable portion of the population, whereas natural mortality is applied to both components. Therefore

$$(5) \qquad N_{j+1} = [(V_j)\,e^{-(F)} + R_j]\,e^{-(M)}$$

The calculated proportion of each age class vulnerable to either estimated mortality rate was year specific (described above). Using this approach, only two instantaneous mortality parameters (representing fishing and double-crested cormorant predation) were estimated in the model, but the influence of both estimated instantaneous mortality parameters on each age class changed over time in relation to observed fish size.

Relative selectivity of gillnet mesh sizes

For comparisons with age-specific estimates of gillnet catchability produced by the estimation model, we calculated relative selectivity curves for walleye based on length distributions of more than 2145 walleye captured in different mesh sizes of the Oneida Lake gill nets (for 1986–1992; data from Forney et al. 1994). We assumed that the maximum selectivity is the same for each mesh size. Maximum selectivity has often been found to occur at a constant ratio of fish girth to mesh perimeter (Hamley 1975). When fish girth is a linear function of length (as is the case for walleye; Henderson and Wong 1991; Forney et al. 1994), this is equivalent to assuming that the modal fish length (the length of maximum retention probability) is a linear function of the mesh size; therefore, the selectivity curves can be es-

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timated directly from the length distribution. Following Hamley (1975) and Wulff (1986), we used a skew normal curve to estimate the length-based relative selectivity of each mesh size for each length bin:

$$(6)\qquad S_{l,m} = \exp\left(-\frac{\lambda_{l,m}^2}{2}\right)\left[1-\frac{k}{2}\sigma_m^{3/2}\left(\lambda_{l,m}-\frac{\lambda_{l,m}^3}{3}\right)\right]$$

where relative selectivity ($S_{l,m}$) was determined for forty-eight, 1 cm length bins (l) ranging from 10 through 57 cm for each of the six mesh sizes (m, as bar mesh in mm). In eq. 6, k is an estimated skewness constant and

$$(7)\qquad \lambda_{l,m} = \frac{L_l - L_{0.5,m}}{\sigma_m}$$

where L_l is the length associated with the lth length bin (cm), and both the modal length of fish captured by mesh size m ($L_{0.5,m}$, total length in mm) and the standard deviation of that mesh (σ_m) are linear functions of the mesh size:

$$(8)\qquad L_{0.5,m} = a + bm$$

$$(9)\qquad \sigma_m = cm$$

where parameters a, b, and c are constants. The four parameters (a, b, c, and k) were estimated using a maximum likelihood function (Wulff 1986) to be 3.2, 0.97, 0.13, and 0.19, respectively.

Only length bins with more than 10 fish were included in the likelihood calculations. These calculations produced a selectivity curve for each of the mesh sizes, which were then summed to produce an overall selectivity curve for the Oneida Lake gill net. Because we assumed the height of the selectivity curves to be constant, this combined curve does not account for size-based changes in swimming speed or activity. Actual selectivity for a given size group of Oneida Lake walleye in the combined standard gill nets also includes a probability of an individual encountering and contacting the net (see Rudstam et al. 1984 or Anderson 1998). This encounter probability may be size dependent.

Results

Indices of walleye abundance

Gillnet effort was constant for all 45 available years, and the annual total catch of walleye in gill nets ranged from 75 to 836. The average percentage of the annual total gillnet catch of walleye that was aged was 96% (range = 66%–100%). Although the catch of age-1 walleye in gill nets was typically lower than that for older ages of a cohort, strong year classes of walleye typically produced large catches at age-1. Trawl effort ranged from 113 to 272 hauls per year (mean = 153) from 1961 to 2003. Trawl CPUE typically declined as the cohort aged, and catches were highest for age-1 walleye for 29 of 37 complete cohorts (data for age-1 to age-7).

Mark–recapture techniques produced age-specific absolute abundance estimates for adult walleye (age-4 and older) for 21 nonconsecutive years. Based on the mark–recapture estimates, the lowest density of adult walleye occurred in 1974; however, the five most recent surveys (performed in 2-year intervals since 1995) produced the next five lowest density estimates for the adult population.

Parameter estimation

The estimation model produced parameter values for the catchability of an active and a passive sampling gear, walleye mortality, and density by age group in the absence of fishery harvest data (Table 2). Three long-term indices of walleye density were synthesized to estimate these previously unknown parameters for walleye in Oneida Lake and aided our understanding of the development of the population over time and our sampling of it.

Age-specific estimates of trawl catchability decreased from age-1 to the aggregate age-8 and older category (Fig. 1*a*). The estimated values for gillnet catchability were lowest for age-1 and peaked for ages 4–6 (Fig. 1*b*). To assess the effects of the weighting coefficients applied to the input data sources, the model was rerun with each input data (gillnet catch-at-age, trawl CPUE, and adult density) set allowed to dominate the objective function. The plotted forms of the estimated age-specific catchability values for both the gill net and trawl were approximately the same but shifted upward for most age classes when more weight was alternatively given to the gear-specific data sets (Fig. 1). Likewise, the catchability estimates for adult walleye were unchanged when the calculated value for natural mortality was either halved or doubled. However, the catchability values for young walleye were more sensitive to this natural mortality value (Fig. 1). To serve as additional reference points for comparison with model estimates, catchabilities of adult walleye in gill nets and trawls were also calculated using the absolute abundance measures from mark–recapture of older walleye. These values also suggested a decreasing trend in catchability of walleye with age in bottom trawls and in gill nets from age-4 to older fish (Fig. 1). For gill nets, these direct calculations were based on the total annual catch-at-age of adult walleye collected at the 15 standard sites and should not be expected to range between 0 and 1 because the annual gillnet survey samples an unknown area of the lake. These decreasing trends in calculated gillnet catchability were expected, in part, because older fish are reaching sizes larger than the peak size selectivity of the largest mesh sizes used in our survey nets.

All ages were affected by the same fixed natural mortality rate. We allowed the instantaneous fishing and cormorant mortality parameters to be functions of fish size and size limits. The model estimated an annual instantaneous fishing mortality rate of 0.327 on vulnerable age classes. Depending on the size limit imposed in different years, the influence of this fishing mortality rate varied. As an example, the average fishing mortality affecting age-5 walleye was 0.327 before the 15-in size limit was put into place (1975), compared with 0.228 when the size limit was 15 in and 0.089 when the size limit was 18 in. Double-crested cormorants were not present on Oneida Lake in large numbers until the early 1990s; therefore, we assumed that walleye in the model were not vulnerable to the mortality parameter reflecting double-crested cormorants until after 1990. During this period, the instantaneous cormorant mortality rate was estimated to be 0.347 on fully vulnerable age-1 fish, while only a portion of other ages were considered vulnerable based on

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Table 2. Log_e-scale parameter estimates and associated linear approximations of the standard deviations shown in parentheses for walleye (*Sander vitreus*) densities and age-specific gear catchabilities.

(*a*) Age-1 density.

	Year									
Decade	xxx0	xxx1	xxx2	xxx3	xxx4	xxx5	xxx6	xxx7	xxx8	xxx9
1958, 1959	—	—	—	—	—	—	—	—	3.67 (0.05)	1.80 (0.18)
1960s	3.37 (0.09)	3.11 (0.13)	2.85 (0.12)	3.16 (0.13)	3.12 (0.1)	3.59 (0.07)	2.97 (0.14)	1.11 (0.2)	2.66 (0.16)	3.58 (0.07)
1970s	–0.06 (0.24)	1.43 (0.19)	3.99 (0.05)	–0.13 (0.23)	0.99 (0.2)	0.80 (0.2)	3.43 (0.17)	0.55 (0.22)	3.64 (0.16)	1.65 (0.19)
1980s	1.30 (0.19)	2.94 (0.15)	3.60 (0.1)	2.61 (0.15)	2.38 (0.14)	2.50 (0.12)	2.49 (0.14)	1.22 (0.19)	3.68 (0.15)	1.44 (0.21)
1990s	1.84 (0.24)	2.00 (0.23)	3.55 (0.2)	1.43 (0.25)	2.23 (0.23)	2.03 (0.24)	2.42 (0.22)	1.59 (0.23)	2.29 (0.22)	2.50 (0.22)
2000–2003	2.51 (0.22)	2.21 (0.3)	3.12 (0.35)	2.39 (0.49)	—	—	—	—	—	—

(*b*) Initial density.

Year	Age-2	Age-3	Age-4	Age-5	Age-6	Age-7	Age-8+
1958	1.5 (0.21)	1.16 (0.26)	3.73 (0.03)	–0.99 (0.49)	1.98 (0.14)	0.42 (0.53)	1.39 (0.25)

(*c*) Age-specific catchability (q_x).

Gear type	Age-1	Age-2	Age-3	Age-4	Age-5	Age-6	Age-7	Age-8+
Gill net (q_G)	0.65 (0.12)	1.45 (0.11)	1.88 (0.11)	1.98 (0.1)	1.84 (0.1)	1.98 (0.1)	1.74 (0.11)	1.61 (0.11)
Trawl (q_T)	–1.13 (0.13)	–1.50 (0.13)	–1.66 (0.12)	–1.89 (0.12)	–1.99 (0.12)	–2.06 (0.13)	–2.22 (0.14)	–2.60 (0.14)

Note: All parameters were originally estimated on a log, scale. The values for log, density are presented first by decade for age-1 fish and then for the remaining ages for 1958 only (the initial year included in the analysis). Next, log, age-specific (age-1 through 8+) catchability values are shown for both the gill net (q_G) and trawl (q_T). Log, instantaneous mortality estimates (linear approximations of the standard deviations in parentheses) associated with fishing (F) and double-crested cormorants (C) are as follows: F = –1.12 (0.03); C = –1.06 (0.18).

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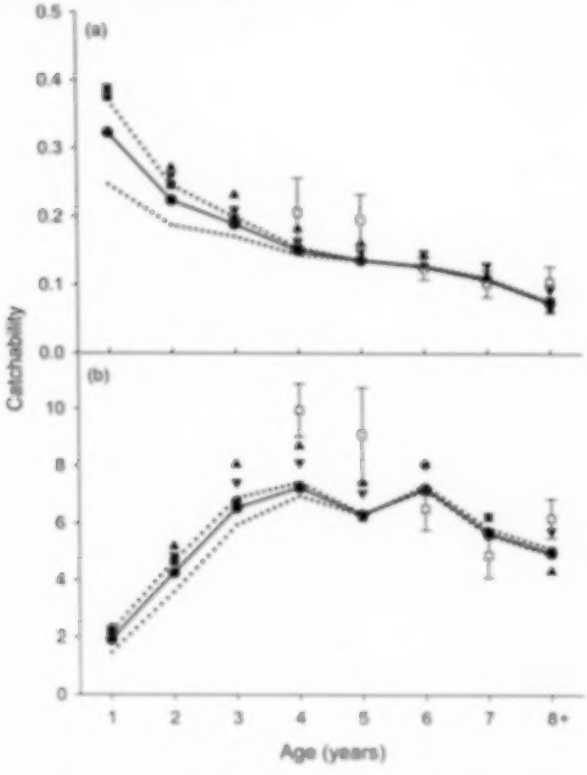
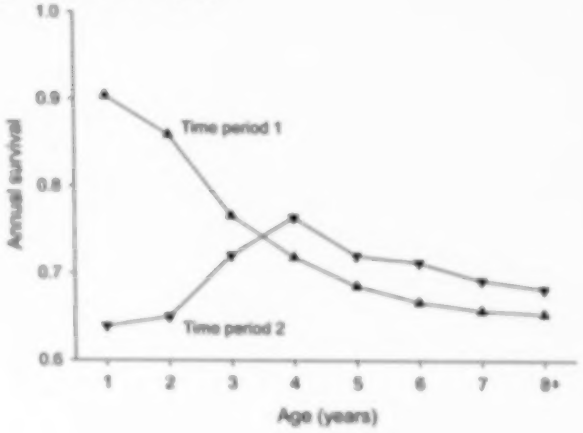
Fig. 1 Age-specific catchability estimates of walleye (*Sander vitreus*) for the (*a*) trawl and (*b*) gill net. See Materials and methods for definition of catchability. Gillnet estimates are based on annual sampling at 15 locations. Circles connected with solid lines indicate base-model estimates. Additional symbols represent modified conditions where the influence of each input data set on parameter estimation was increased by a factor of 10 (■, weighted towards mark–recapture; ▲, weighted towards gill nets; ▼, weighted towards trawls). The upper broken line represents the catchability estimates using half of the original estimate for natural mortality; the lower broken line represents the catchability estimates using twice the original estimate for natural mortality. For an additional comparison of scale with model estimates, average age-specific catchability values calculated directly for adult walleye (□; ±1 standard error) are shown for both gears.



their total length (effective instantaneous cormorant mortality averaged 0.330, 0.205, and 0.058 for ages 2, 3, and 4, respectively). Very few age-5 or older walleye were expected to be vulnerable to cormorant mortality in our model. Both instantaneous fishing and instantaneous cormorant mortality were additive to the fixed instantaneous natural mortality rate (0.101). Annual survival estimates for walleye were quite different for the two time periods (before and after 1990; Fig. 2) because of both the additional cormorant mortality and implementation of an 18-in size limit in recent years. No single age class of walleye was considered fully vulnerable to both fishing and cormorant mortality.

The model estimated an initialization value for walleye density (9 walleye·ha^{-1}). Annual recruitment (at age-1) devi-

Fig. 2 Age-specific estimates of annual survival of walleye (*Sander vitreus*) averaged for both time period 1 (1958–1989, ▲) and time period 2 (1990–2003, ▼).



ations were applied to this density estimate to initiate new year classes in the model. A lower number of age-1 walleye (associated with higher mortality between ages 0 and 1) likely contributed to the overall decline seen in our catches. The highest annual adult density occurred during the first year included in the model (1958) because of the presence of a large number of age-4 walleye; however, later cohorts did produce higher densities at younger ages (Fig. 3). Since 1990, the average densities of both subadult (ages 1–3) and adult (age-4 and older) are barely half of earlier average densities (Fig. 4).

Relative selectivity of gillnet mesh sizes

We calculated relative selectivity for the individual gillnet mesh sizes based on walleye length distributions external to the estimation model (see Materials and methods). Because gillnet catchability values from the estimation model were age-specific, we assigned length-at-age values based on estimated growth rates and back-calculated length-at-age estimates using all available data (1958–2002) so that the model estimates could be visually compared with size-based relative selectivity patterns (Fig. 5). The gillnet catchability estimates from the estimation model increased slightly faster with walleye length from ages 1 to 6 than the selectivity derived from catches in the different mesh sizes.

Discussion

We combined multiple sources of information and used a minimal number of limiting assumptions to estimate mortality and age-specific density of age-1 and older walleye in Oneida Lake as well as age-specific catchability values for both the standard gill net and trawl. This approach simultaneously used weighted contributions from three data sets (gill net, trawls, and adult mark–recapture), which were collected over a 46-year period, to construct an internally consistent picture of the development of the walleye population over time in the absence of annual fishery effort and harvest data.

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Fig. 3 Bubble plot representing age-specific density estimates of walleye (*Sander vitreus*) from 1958 through 2003.

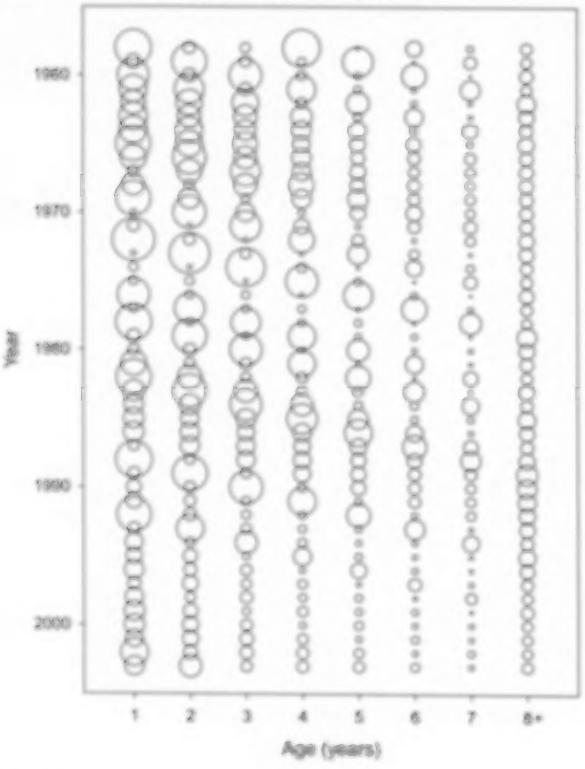
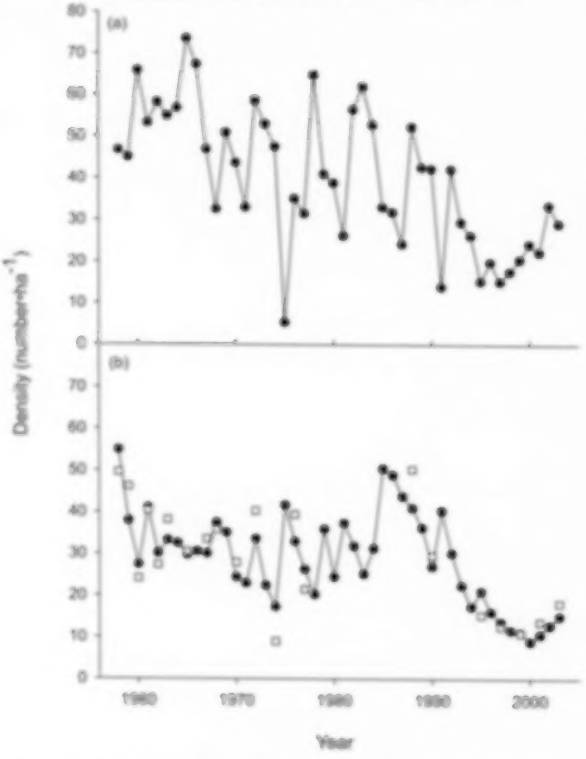


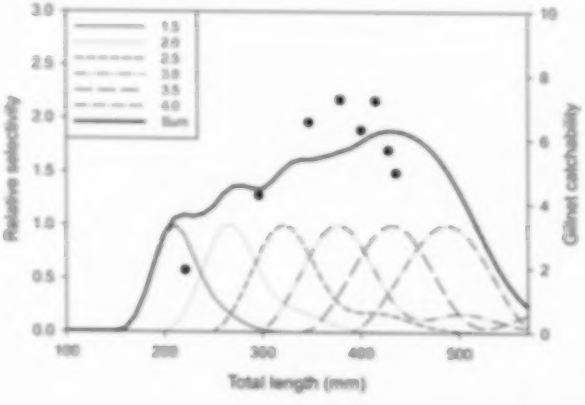
Fig. 4 Estimated density of (*a*) subadult (age-1 through age-3) and (*b*) adult (age-4 through age-8+) walleye (*Sander vitreus*) in Oneida Lake from 1958 through 2003. Adult mark–recapture estimates are shown for comparison for the years they were available (□).



Mortality

During the earlier years, mortality of subadult walleye was primarily natural mortality, although these young fish occasionally were large enough to be affected by fishing mortality. As a result, estimated average annual survival of age-1 walleye during the initial time period (1958–1989) was high (90.4%) but fell to the lowest observed average annual survival estimate (63.9%) during the second time period (1990–2002) when this age was also affected by the additional mortality parameter. As such, the average annual survival of age-1 walleye showed the greatest change of all the represented age classes between the two mortality time periods. The average annual survival of age-2 walleye in the first time period (86.0%) was slightly lower than that for age-1 fish because length-at-age values suggested a small proportion of age-2 walleye would have been vulnerable to angler harvest during the first time period, when some years had no length-based harvest restrictions (we assumed a 12-in minimum harvest size for these years). Similar to age-1 fish, the average survival of age-2 walleye was lower during the second time period, as these fish were influenced by the double-crested cormorant mortality parameter. Previously, Rudstam et al. (2004) observed that mortality of subadult walleye in Oneida Lake had increased and suggested that this was a result of increased predation by double-crested cormorants. Results of our estimation exercise support this hypothesis. Rudstam et al. (2004) used only the trawl data as an index of age-1 abundance and

Fig. 5 Estimated age-specific gillnet catchability (●) for walleye (*Sander vitreus*) in Oneida Lake and a relative selectivity curve based on the catch of walleye in different mesh sizes. The relative selectivity curve is the sum (thick solid line) of the individual mesh size curves (thin solid and broken lines). Gillnet mesh sizes ranged from 1.5 to 4 inches in half-inch increments (1 in = 25.4 mm). The catchability-at-age values estimated by the model were assigned length-at-age values, based on estimated growth rates and back-calculated length-at-age estimates (1958–2002), so that the model estimates could be plotted against the size-based relative selectivity curve.



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only mark–recapture data combined with trap-net catches to estimate age-4 abundance. Here, we simultaneously used three long-term, fishery-independent data series that contain separate, age-specific information and confirmed high mortality of subadult walleye after 1990. Although this does not directly identify the causative source of this additional mortality, we also suggest that cormorants were the most likely source of increased mortality for subadult walleye (see Rudstam et al. 2004).

The overall changes in average survival of ages 3 and 4 walleye between time periods were much less than that for the youngest two age classes. This was due in part to these ages typically being less vulnerable to predation from double-crested cormorants and to increased length limits, offering protection of fish between 15 and 18 inches, during the second time period when double-crested cormorants increased in abundance. Overall, the two estimated mortality parameters included in the model suggested mortality additional to the fixed natural mortality level, although the actual influence of these two additional parameters varied among ages. To facilitate calculation of natural mortality, we used data from a few years that included measures of angler harvest. We believe our estimate of a relatively low natural mortality for all age groups is reasonable because mortality of age-1 walleye in Oneida Lake is expected to be primarily due to predation, and most of this mortality likely occurred prior to our sampling; however, we did explore model sensitivity to this fixed value by modifying the natural mortality rate by factors of 0.5 and 2. During our sampling period, age-1 walleye would have outgrown some gape-limited predators, and age-0 fishes typically dominate the diets of adult walleye (Chevalier 1973; Forney 1974). Double-crested cormorants are the only predator in the lake known to select for age-1 walleye.

For age-5 and older walleye, the applied instantaneous fishing mortality (F) ranged from 0.03 to 0.3 depending on observed fish size and the size limits imposed during a given year. This range of mortality is similar to previous calculations from Oneida Lake (VanDeValk et al. 2002, 2005*b*) but is not a particularly high fishing mortality — for a single season, annual mortality from fishing can approach 50% ($F = 0.78$; Forney 1967). However, our model did not allow fishing mortality in individual years to be higher than the estimated value of full vulnerability to the fishery. Angler harvest can be highly variable, as evidenced by a comment by the Editor of *Conservationist* in 1961: "Oneida Lake fishermen—and they are legion—have long tried to guess why walleyed pike fishing, for which the lake is famous, could be so good one year, almost a waste of time the next" (see Forney and Eipper 1961). For a given year, angler catch rates in Oneida Lake is primarily determined by the availability of forage fish to walleye and largely unrelated to walleye abundance (Forney and Eipper 1961; Forney 1965; VanDeValk et al. 2005*a*). Even so, adult walleye survival during recent years should have benefited from more restrictive harvest regulations, which were in place during years when double-crested cormorant mortality was affecting subadults. For Oneida Lake, exploitation by anglers has been measured for only a few years, and because of this lack of long-term information, we did not attempt to directly include variable angler harvest in this estimation exercise. However, if harvest is known, then this information could be included in a similar estimation model.

Age-specific gear catchability

Fish populations are often sampled using both passive and active gears, but catch rates of these gear types are not directly comparable unless the catchabilities are known. The problems associated with comparing or combining data from the two sampling approaches generally arises from the difficulty in quantifying the sampling area of the passive gear; however, both gear types provide information on the size of the population. Here, the choice of weighting coefficients used to regulate the influence of each input data set on the estimates had relatively little influence on the catchability estimates. The mark–recapture procedures offered the most direct measure of absolute abundance for Oneida Lake walleye. Although we have confidence in these mark–recapture estimates of adult walleye abundance (confidence intervals are typically ±20%), this input data set contained no direct estimates for subadults and had no estimates for many of the years that the population was sampled with both the trawl and gill net. The two walleye collection gears likely have different age biases; trawl data are likely better than gill nets to serve as an indicator of subadult abundance, while gill nets are likely better than trawls as an indicator of adult abundance. We had no basis for initially weighting one data set more than the other and felt that giving equal weight to all three long-term input data series was the most appropriate assumption.

Our model estimates did include the assumption that age-specific gear catchability has not changed in Oneida Lake over the study period. This assumption is reasonable because the sampling protocols and gears have been standardized on Oneida Lake for nearly half a century. This is in contrast with many organized commercial fisheries where technological advancements can lead to increased catchability over time. However, caution is necessary when applying the assumption of constant catchability over time, even for standardized sampling (Gordoa and Hightower 1991). Catchability may also respond to fluctuations in abundance (Peterman and Steer 1981) and to changes in environmental conditions, such as temperature, oxygen levels, or water clarity (Nielsen 1983). Additionally, ecological changes may indirectly affect catchability by influencing encounter probabilities of various gears if the growth rates, foraging behaviors, or preferred habitats of the target species are altered (Henderson and Wong 1991). Although it has been shown elsewhere that neither abundance nor environmental conditions have had strong effects on the gillnet catchability of adult walleye in Oneida Lake (J.R. Jackson, unpublished data), this may not be the case for trawl catches, as water clarity has increased in the lake since the arrival of zebra mussels (Idrisi et al. 2001).

The relative size selectivity of gill nets can also be estimated from the length distributions of fish captured in different mesh sizes (Hamley 1975). For Oneida Lake, we used the length distribution of Oneida Lake walleye captured in different mesh sizes to construct relative selectivity curves where the modal length and standard deviation of the selectivity curve were each a linear function of mesh size and the relative selectivity at the modal length of each

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mesh size were assumed to be equal. The combined selectivity of this set of gill nets generally increased to 440 mm because the larger walleye may be caught in more than one mesh size. The catchability of larger fish may actually be higher than predicted from this curve because large mesh may be less visible than small mesh (Hamley 1975) or because larger fish may have a higher encounter rate (Rudstam et al. 1984) or become more easily entangled (Grant et al. 2004). Hamley and Regier (1973) found substantially higher catchability for larger walleye in a study where a known population was fished with experimental gill nets. Our model results generally support the shape of the relative selectivity curve. Although our estimates of catchability increased slightly faster than was predicted by this curve, the increase was less than predicted by the Hamley and Regier (1973) selectivity curves. Rudstam et al. (1984) suggested that encounter probability should increase with fish length to approximately the 0.5 power based on an argument of length dependence of routine swimming speed for several fishes. For Oneida Lake walleye, an increase in encounter probability with fish length to the 0.3 power was sufficient to account for the difference in slope between the estimation model results and the relative selectivity of the nets. This suggests that accounting for increased catchability of larger walleye is less important than suggested by Hamley and Regier (1973; see also Henderson and Wong 1991 and Grant et al. 2004).

Compared with studies using gill nets, less attention has been given to assessing freshwater trawl selectivity. Consideration of indirect selectivity of towed gears may involve use of covered cod ends, trouser trawls, twin-gear, or alternate hauls using nonselective gear variants (Millar and Fryer 1999). Generally, larger individuals may possess greater physical ability to escape the path of the gear. Olin and Malinen (2003) found that large (>19.9 cm) European perch (*Perca fluviatilis*) and zander (*Sander lucioperca*) were under-represented in trawl catches relative to gill nets. Our estimated values for trawl catchability also suggest walleye are captured with reduced efficiency at larger sizes. Because we were interested in temporal changes in mortality, we could not assess the concurrent changes in water clarity or light penetration on trawl catchability, although vision also likely plays an important role in gear avoidance (Glass and Wardle 1989). Note, however, that a decrease in catchability due to increased water clarity in the 1990s would increase the additional mortality required between ages 1 and 4 to explain the current decrease in cohort strength at age-4. Our observations of increased subadult mortality discussed above are therefore conservative against potentially unaccounted for decreased catchability in the trawl (or gill nets).

In summary, our estimation model simultaneously used three long-term data sets to estimate walleye density, mortality, and catchability associated with the standard trawl and gillnet surveys. Although fishery catch data are commonly incorporated into stock assessments, we performed this estimation exercise without annual information of angler harvest. Even under the restrictive constraints placed on the mortality parameters, the model produced age-specific catchability estimates of the anticipated form for both gill net and trawl. Our final estimate of walleye population structure is generally congruent with earlier estimates for adult walleye, which were based mostly on mark–recapture and a trap-net index (e.g., Mills and Forney 1988; Rudstam et al. 2004). The current model uses all three available long-term data series and, in contrast with earlier calculations, consistently produces realistic declines in walleye abundance with age. Likewise, the model followed the high variation in walleye year-class strength, with the maximum estimated density of age-1 fish more than 50 times larger than the lowest density estimated for this age class. Here, the highest estimated density for an adult age class occurred during 1958 for age-4 walleye, and this is consistent with previous measures of the strong 1954 year class moving into the adult population (Forney 1967). The model estimates suggest a strong reduction in average annual survival of subadult walleye in Oneida Lake after 1990 in accordance with hypothesized predation effects of double-crested cormorants (e.g., Rudstam et al. 2004). However, data limitations and imposed constraints on mortality do not allow this model to directly identify year-to-year changes in mortality caused by variable harvest rates. Overall, the use of multiple long-term data sets allowed for the estimation of several population parameters of interest and improved our understanding of the development of the walleye population over time and our sampling of it, even though a time series of more traditionally used fishery catch data was not available for this study.

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Among- and within-tributary responses of riverine fish assemblages to habitat features[1]

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Abstract: We quantified fish abundance and environmental variables at 170 sites distributed among 11 tributaries of the Ottawa River, Quebec, Canada, to assess the relative importance of among- and within-tributary variation in riverine fish assemblages. Additionally, we determined (*i*) which environmental variables were most strongly associated with each type of variation and (*ii*) whether ecomorphological traits in fish assemblages were predictably related to environmental gradients. Partitioning of variation by means of partial ordination indicated that assemblages were less variable among (38.7% of the total variation) than within (61.3%) tributaries. Water transparency singly accounted for 33.3% of the variation among tributaries, whereas macrophyte cover and river width jointly accounted for 8.3% of the variation within tributaries. These results suggests that differences in habitat features among tributaries may account for a substantial fraction of the predictable variation in assemblage structure at the watershed scale, an aspect not emphasized in previous studies of riverine fish assemblages. Mixed regression analyses relating ecomorphological traits to environmental variables showed that the environmental variables most strongly associated with assemblage structure were significantly related to traits associated with predator avoidance or foraging efficiency.

Résumé : Les communautés de poissons et des variables environnementales ont été quantifiées à 170 sites répartis le long de 11 affluents de la rivière des Outaouais (Québec, Canada), afin de déterminer l'importance relative de la variation inter- et intra-affluents dans la structuration des communautés. De plus, nous avons déterminé (*i*) quelles variables environnementales étaient le plus fortement associées à chaque type de variation et (*ii*) si les traits écomorphologiques caractérisant les communautés étaient liés de façon prévisible aux gradients environnementaux inter- et intra-affluents. La partition de la variation par ordination partielle a mis en évidence que les communautés étaient moins variables entre elles (38,7 % de la variation totale) qu'au sein (61,3 %) des affluents. La transparence de l'eau expliquait à elle seule 33,3 % de la variation entre les affluents, tandis que le couvert de macrophytes et la largeur de la rivière expliquaient conjointement 8,3 % de la variation au sein des affluents. Ces résultats suggèrent que des différences dans les caractéristiques de l'habitat entre les affluents pourraient jouer un rôle prépondérant dans la structuration des communautés de poissons de rivière à l'échelle du bassin versant, un aspect qui n'a pas été mis en lumière dans les études précédentes. Des analyses de régression mixte mettant en relation les traits écomorphologiques et les variables environnementales ont montré que les variables environnementales associées à la structuration des communautés étaient significativement reliées à des traits liés à l'évitement des prédateurs et à l'efficacité de l'alimentation.

Introduction

Variation in riverine fish assemblages within a watershed can be conceptually split into among- and within-tributary components. Apportioning assemblage variation to among- and within-tributary components and identifying the environmental determinants of both types of variation may have important implications for riverine fish ecology, yet very few studies have specifically quantified the relative importance of these two components (e.g., Humpl and Pivnička 2006). For example, when species–environment relationships are stronger among than within tributaries, differential colonization of tributaries from the river mainstem (including both immigration to the tributary and subsequent establishment in it) may be driven by habitat preferences of the species in the regional pool. Assemblage structure and local adaptations by individuals may then be primarily regulated by transversal (i.e., among-tributary) processes. Exchanges between tributaries and the river mainstem can be of great consequence to the organization of fish assemblages at the local and watershed scales, as suggested by the network dynamics hypothesis (Benda et al. 2004; Lowe et al.

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2006). This hypothesis views rivers as networks of channels and confluences and proposes that the physical characteristics at tributary junctions can intensify spatial and temporal habitat heterogeneity at those points, thereby influencing the productivity and distribution of aquatic organisms (e.g., by generating biological hotspots). The importance of exchanges between tributaries and the mainstem is also supported by evidence from field studies showing that tributaries located lower in a drainage network have greater species richness than similarly sized streams located in the headwaters of the network (Osborne and Wiley 1992) and that mainstem species richness tends to increase downstream of tributary confluences (Cox Fernandes et al. 2004).

In contrast, when species–environment relationships are stronger within than among tributaries, species from the river mainstem may not preferentially colonize specific tributaries, and assemblage structure at the watershed scale, as well as local adaptations by individuals, should be related mostly to longitudinal processes (Rahel and Hubert 1991; Reyjol et al. 2001; Grenouillet et al. 2004). Change in fish assemblage structure within tributaries is often predictably related to longitudinal gradients in environmental variables such as water temperature, water transparency, current velocity, dissolved oxygen concentration, and substratum size (e.g., Ostrand and Wilde 2002; Li and Gelwick 2005; Humpl and Pivnička 2006). Elucidating the rules underlying fish assemblage organization at the watershed scale can contribute to our understanding of evolutionary processes, because watersheds can be biogeographical stages for speciation and extinction events (Reyjol et al. 2007).

Aquatic ecologists are interested in relating not only the taxonomic composition of fish assemblages, but also their traits composition (i.e., the relative representation of key biological or morphological attributes) to environmental variation (Goldstein and Meador 2004; Hoeinghaus et al. 2006). Studies examining relationships between morphology and habitat often focus on feeding ecology in the context of resource partitioning (e.g., Winemiller 1991); relatively few studies have examined the responses of morphological traits of riverine fish species to environmental variation (e.g., Gatz 1981). However, morphological traits have been shown to respond to both hydrological and geomorphic gradients (Lamouroux et al. 2002; Willis et al. 2005) and to vary in relation to river size (i.e., from small streams to large rivers; Goldstein and Meador 2004).

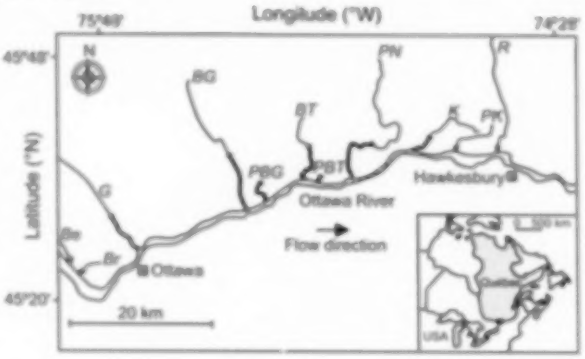
The objectives of the present study were to determine (*i*) the relative importance of among- and within-tributary variation in fish assemblages of the Ottawa River, Quebec, Canada; (*ii*) which environmental variables were most strongly associated with each type of variation; and (*iii*) whether ecomorphological traits of fish assemblages were predictably related to environmental gradients both among and within tributaries.

Materials and methods

Study area and sampling methods

Fish samples and environmental measurements were collected during the summer in 1995 (31 July – 8 August) and 1996 (8–23 August) from 11 tributaries spread across a 70 km stretch of the Ottawa River (watershed area: 146 000 km^2; mean discharge: 1937 m^3·s^{-1}) (Fig. 1). Along this stretch, the alluvial plain of the Ottawa River is generally narrow (several hundred metres to several kilometres); its sedimentary deposits are rich in clay and silt. The tributaries originate in the Canadian Shield and run through it before traversing the alluvial plain and reaching the Ottawa River. In the reaches running through the shield, the tributaries have clear waters and streambeds characterized by coarse substrata, but transparency declines as the tributaries enter the plain. The extent of this decline varies among tributaries because the decline arises from loading of fine inorganic sediment, which in turn depends on the length of the trajectory through the plain (H. Fournier, Ministère des Ressources naturelles et de la Faune, Direction de l'aménagement de la faune de l'Outaouais, Faune Québec, 98 rue Lois, Gatineau, QC J8Y 3R7, Canada, personal communication).

Fig. 1. Tributaries of the Ottawa River included in the present study: Be, Bélisle Brook; Br, Breckenridge Brook; G, Gatineau River; BG, Blanche à Gatineau River; PBG, Petite Blanche à Gatineau River; BT, Blanche à Thurso River; PBT, Petite Blanche à Thurso River; PN, Petite Nation River; K, Kinonge River; PK, Petite Kinonge River; R, Rouge River. In each tributary, samples were collected at equidistant points along the reaches (thick tracing) between the Ottawa River and the first insurmountable barrier (hatch mark).



Within each tributary, systematic sampling was conducted at sites spaced uniformly between the Ottawa River and the first barrier judged to be insurmountable to fish. Barriers were generally falls or short rapids associated with an escarpment several metres high at the junction of the river plain with the Canadian Shield. In all, 170 sites were sampled, 10 in each of the five tributaries for which the barrier was <1 km away from the Ottawa River and 20 in each of the six remaining tributaries (Table 1). Fish were collected at the selected sites using a beach seine (length: 20.3 m; height: 3.7 m at the center and 1.8 m at the extremities; 12.7 mm stretch mesh). At each site, one seine haul was conducted over a semicircular area (~160 m^2) free of obstacles to effective seining (e.g., steep slope, snags). Fish were counted and identified to species. Nine environmental variables were measured (Table 2): river width, bank slope, water transparency (Secchi depth), mean current velocity (Gurley Price current meter), dissolved oxygen concentration (YSI 54 A), pH (Brinkmann E604), macrophyte cover (bed width), macrophyte richness (number of taxa in the

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Table 1. Code, number of sampling sites, and distance between the Ottawa River and the first barrier on the tributary for the 11 tributaries of the Ottawa River included in this study.

Tributary	Code	No. of sites	Distance (m)
Bélisle	Be	10	630
Blanche à Gatineau	BG	20	7700
Blanche à Thurso	BT	20	6200
Breckenridge	Br	10	600
Gatineau	G	20	5900
Kinonge	K	20	3100
Petite Blanche à Gatineau	PBG	20	3000
Petite Blanche à Thurso	PBT	10	790
Petite Kinonge	PK	10	820
Petite Nation	PN	20	8200
Rouge	R	10	730

seined area; resolution to species or genus), and a substratum size index, defined as the most common size class in the seined area, as determined by visual and tactile examination (size classes were coded as scores ranging from 1 to 6 for particle diameters with boundaries at 1, 3, 17, 65, and 265 mm; modified from Bovee 1986). River width and macrophyte cover were measured along a transect perpendicular to flow, corresponding to a cross-section over the whole river width. Water transparency, mean current velocity, dissolved oxygen concentration, and pH were measured at the centre of the transect. Mean current velocity was measured at 0.6 times water depth for depths <1 m and at 0.2, 0.6, and 0.8 times water depth for depths >1 m (Bovee 1986). In the latter case, mean current velocity was calculated as $V_{mean} = (V_{0.2} + 2V_{0.6} + V_{0.8})0.25$. Bank slope was calculated from the horizontal distance, measured transversally to flow, between the shoreline and the point at which water depth attained 3 m. For two shallow tributaries (Bélisle Brook and Breckenridge Brook), a depth of 0.5 m was used instead of 3 m.

Partitioning of variation and species–environment relationships

We conducted three separate ordination analyses: a full analysis including both among- and within-tributary variation, equivalent to a conventional ordination; and two partial analyses, one focusing on among-tributary variation and the other on within-tributary variation. In the full analysis, variation in assemblage structure was examined in relation to a set of environmental predictors, without using covariates. The partial analyses were used to apportion the total variation in fish assemblage structure into among- and within-tributary components and to quantify, for each component, the variation explained by the environmental variables. Three sets of covariates were used in the partial analyses (see simplified example in Appendix A, Table A1). The first set of covariates, **T**, was structured as a 170 × 10 (rows × columns) matrix of effects coding that assigned each of the 170 samples to one of the 11 tributaries (only 10 variables are required to distinguish among 11 tributaries). Covariate set **T** accounts for variation among tributaries. The second set of covariates, **S**, was structured as a 170 × 19 matrix of effects coding that assigned each of the samples to a specific site within the tributary (19 variables are required to distinguish among 20 sites). Covariate set **S** accounts for additive patterns of within-tributary variation that are shared by all tributaries. The third set of covariates was obtained by first calculating the column-by-column product of **T** and **S** to yield a 170 × 190 matrix (command matrix.model in the R software, v. 2.3.1; R Development Core Team 2006) and then reducing this matrix to a 170 × 140 matrix by eliminating 50 collinear variables (collinear variables are dropped automatically by most linear modelling software; e.g., command lm in the R software). The resulting matrix, **T**·**S**, coded for the interaction between tributaries and sites. Covariate set **T**·**S** accounts for the residual (nonadditive) within-tributary variation that is not shared among tributaries and reflects site-specific deviations from the additive pattern common to all tributaries. The coding used ensures that covariates set **T** is linearly independent of sets **S** and **T**·**S** combined; furthermore, sets **T**, **S**, and **T**·**S** jointly exhaust the variation in assemblage structure. In this context, the interaction term **T**·**S** is used jointly with term **S** solely as a quantitative device to extract all of the within-tributary variation. We were not interested in an ecological interpretation of the interaction term, because this term reflects idiosyncratic variation associated with site-specific characteristics that do not generalize across tributaries.

The total variation in fish assemblage structure was partitioned into an among-tributary component, obtained as the variation accounted for by set **T**, and a within-tributary component, calculated as the total variation minus the among-tributary variation. Equivalently, the within-tributary component could have been obtained directly as the variation jointly accounted for by sets **S** and **T**·**S**, and the among-tributary component could then be calculated as the total variation minus the within-tributary variation.

The variation explained by the environmental variables among tributaries and within tributaries was quantified in two separate analyses. In the first analysis, set **T** was used as covariates in a partial ordination to statistically remove differences among tributaries, thereby focusing on environmental effects related solely to within-tributary variation in assemblage structure. This analysis included a set of envi-

Table 2. Median and upper and lower 5% percentiles for nine environmental variables measured at 170 sites.

Environmental variable	Median (5%–95%)	ICC
River width (m)	26.5 (8.5–260.0)	0.85
Bank slope (°)	13 (2–25)	0.18
Water transparency (cm)	67 (18–293)	0.91
Mean current velocity ($cm \cdot s^{-1}$)	<1.5 (<1.5–7.3)	0.45
DOC (ppm)	7.9 (4.6–8.5)	0.95
pH	7.8 (6.7–8.4)	0.93
Macrophyte cover (m)	3.0 (0.0–19.0)	0.27
Macrophyte richness (no. of taxa)	3 (0–8)	0.31
Substratum size (index)	1 (1–2)	0.17

Note: The intraclass correlation coefficient (ICC), measured as the ratio of among-tributary variation to total variation (among- plus within-tributary), is also reported for each variable. DOC, dissolved organic carbon.

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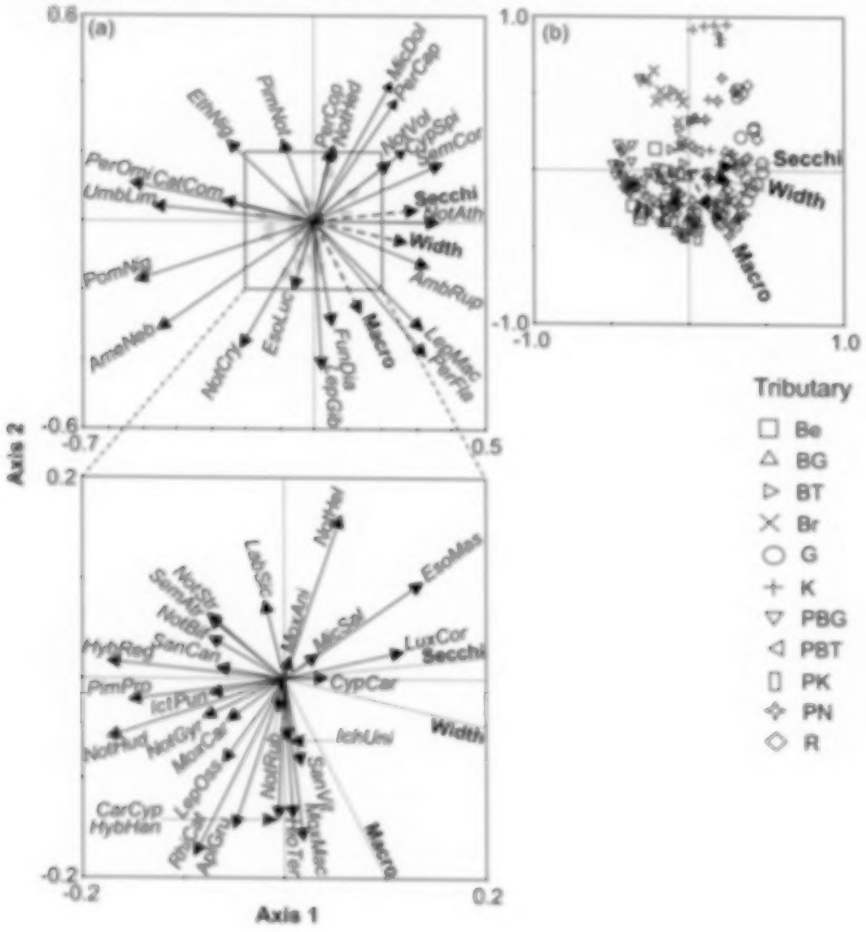
Fig. 2. (*a*) Species and (*b*) sites ordination plots for the full redundancy analysis (RDA). The two ordination axes (axis 1: $P = 0.005$; axis 2: $P = 0.020$) jointly account for 20.4% of the total variation in assemblage structure. Species codes are given in Appendix B, Table B1. Be, Bélisle Brook; Br, Breckenridge Brook; G, Gatineau River; BG, Blanche à Gatineau River; PBG, Petite Blanche à Gatineau River; BT, Blanche à Thurso River; PBT, Petite Blanche à Thurso River; PN, Petite Nation River; K, Kinonge River; PK, Petite Kinonge River; R, Rouge River; Secchi, water transparency; Macro, macrophyte cover; Width, river width.



ronmental variables as predictors in addition to set **T** as covariates. In the second analysis, sets **S** and **T**·**S** were used jointly as covariates in a partial ordination to statistically remove the variation within tributaries and focus on environmental effects related solely to among-tributary variation in assemblage structure. This analysis included a set of environmental variables as predictors in addition to sets **S** and **T**·**S** used jointly as covariates.

We based all ordinations on redundancy analyses (RDA) and partial redundancy analyses instead of the more commonly used canonical correspondence analyses (CCA). CCA did not allow us to unambiguously partition the variation, presumably because rows in the design matrix in CCA are assigned different weights, and therefore linearly independent covariates do not remain independent after weights are applied. In contrast, no such weighting is used in RDA. We used a Hellinger transformation of species abundances because a preliminary detrended correspondence analysis yielded a relatively long gradient (3.97 standard deviations in the sample scores). The Hellinger transformation renders RDA effective as an alternative to CCA for the analysis of species abundances when gradients are long (Legendre and Gallagher 2001). In the ordination analyses involving environmental variables, a forward selection procedure based on a nominal cut-off point ($P = 0.05$) was used to determine which of the nine environmental variables measured were useful predictors of assemblage structure (program CANOCO, version 4.5; ter Braak and Šmilauer 2002). Environmental variables not retained by the selection procedure were not included in the ordinations. Statistical significance was determined by Monte Carlo tests (1000 permutations) for the individual predictors, the sum of all eigenvalues, and the ordination axes. The nested structure of samples was maintained during resampling by use of within-block permu-

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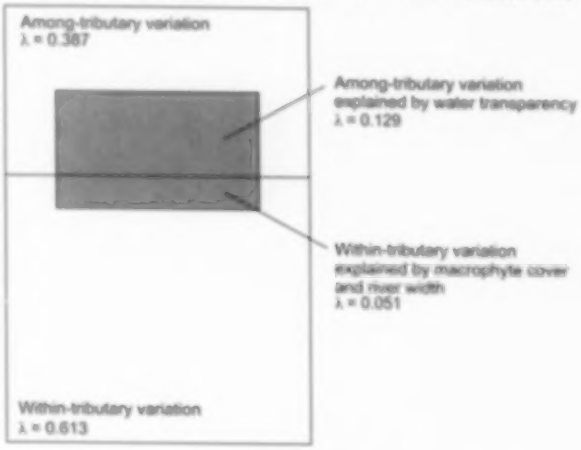
tations (i.e., random reshuffling was unrestricted among tributaries with the same number of sites but was restricted for sites within tributaries).

Influence of environmental variables on ecomorphological traits

Ecomorphological traits potentially linked to ecology and behaviour were obtained for the fish species in this study (Appendix B, Table B1) from published sources, following an approach similar to those of Poff and Allan (1995) and Lamouroux et al. (2002). Image analysis software (SigmaScan Pro, version 5.0; Systat Software Inc., Point Richmond, California) was used to measure six quantitative traits from illustrations in Scott and Crossman (1985): relative eye diameter (standardized by total length; EyeDiam), relative length of the pectoral fin (standardized by total length; PectLength), relative insertion height of the pectoral fin (ratio of insertion height to body depth at insertion site; PectHeight), dorsal insertion distance (snout to anterior insertion of dorsal fin, standardized by total length; DorsDist), shape factor (ratio of total length to maximum body depth; ShapeF), swimming factor (ratio of minimum depth of caudal peduncle to maximum depth of caudal fin; SwimF). Five qualitative traits were coded using information in Scott and Crossman (1985), Froese and Pauly (2006), and the Fish ID collection (Lyons et al. 2006): mouth position (Mouth: 0, inferior; 1, terminal or subterminal; 2, superior); four variables denoted presence or absence of a trait (binary coding): barbels (Barbels), mottled or vertical stripe patterns on the body (Pattern), silvery body coloration (Silver), and lateral stripe (LatStripe). Analyses of PectHeight and SwimF did not include the silver lamprey (*Ichthyomyzon unicuspis*), which lacks pectoral fins and a distinct caudal peduncle. For each site, a mean value was obtained for each of the traits by averaging across all individuals captured at the site, irrespective of species identity.

In nested sampling designs, units within a group tend to be more similar to other units in the same group than to units in other groups (i.e., there is substantial intraclass correlation); thus, individual observations are not entirely independent as required by conventional regression models. To account for the nesting of sites within tributaries, linear mixed regressions (nlme package version 3.1-79 of the R software; R Development Core Team 2006), which account for intraclass correlation by means of random effects for the parameters, were used to assess the relationship between each ecomorphological trait and the environmental variables retained in the partial ordination analyses. A parametric bootstrap (1000 iterations) was used to determine whether random effects were needed for the slope and intercept (Faraway 2006). Because several environmental variables were tested at once in each model, their effects were assessed through Wald tests adjusted by the Benjamini–Hochberg (false discovery rate) procedure (García 2004). The intraclass correlation coefficient (ICC), which measures the proportion of the total variation that is among groups (Faraway 2006), was used to quantify the relative importance of among- and within-tributary variation for each of the environmental variables. The ICC can range from 0 (all variation is within tributaries) to 1 (all variation is among tributaries). Linear mixed regressions were also used to assess longitudinal variation in environmental variables selected in the partial ordinations. In all analyses, logarithmic (river width, water transparency, mean current velocity, macrophyte cover) or square-root (bank slope) transformations were used to reduce the influence of extreme points and better fit the assumptions of linearity, normality, and homoscedasticity.

Fig. 3. Venn diagram illustrating the partitioning of total and explained variation in fish assemblages among and within tributaries. The ordination eigenvalues (λ) reflect the variation associated with each component, expressed as a fraction of the total variation (1.0).



Results

In the full ordination analysis, three of the nine environmental variables initially considered were retained by the forward selection procedure: water transparency (P = 0.002), macrophyte cover (P = 0.001), and river width (P = 0.007). The first ordination axis reflected a gradient in water transparency and river width, whereas the second axis was associated with a gradient in macrophyte cover (Fig. 2*a*). Species associated with water transparency and river width included several small cyprinids (emerald shiner (*Notropis atherinoides*), spotfin shiner (*Cyprinella spiloptera*), mimic shiner (*Notropis volucellus*), fallfish (*Semotilus corporalis*)), rock bass (*Ambloplites rupestris*), bluegill (*Lepomis macrochirus*), yellow perch (*Perca flavescens*) (positive associations); and trout-perch (*Percopsis omiscomaycus*), black crappie (*Pomoxis nigromaculatus*), central mudminnow (*Umbra limi*), and brown bullhead (*Ameiurus nebulosus*) (negative associations). Species associated with macrophyte cover included bluegill, pumpkinseed (*Lepomis gibbosus*), yellow perch, banded killifish (*Fundulus diaphanus*) (positive associations); and bluntnose minnow (*Pimephales notatus*), and johnny darter (*Etheostoma nigrum*) (negative associations). The scatter on the ordination plot for sites (Fig. 2*b*) reflects variation both among and within tributaries.

Partitioning of the total variation showed that assemblages were less variable among (38.7% of the total variation) than within tributaries (61.3%) (Fig. 3). In the partial ordination analysis focusing on variation among tributaries (**S** and **T**·**S** as covariates), water transparency was the only predictor re-

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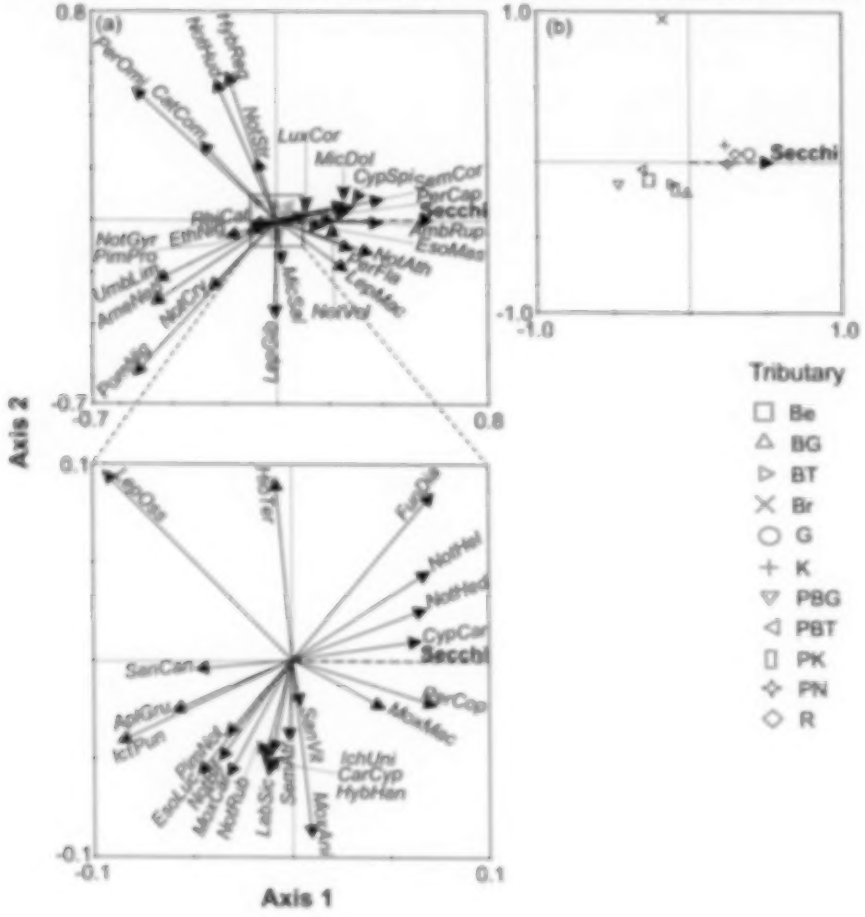
Fig. 4. (*a*) Species and (*b*) sites ordination plots for the partial redundancy analysis (RDA) focusing on among-tributary variation. The first ordination axis ($P = 0.003$) accounts for 33.3% of the among-tributary variation in assemblage structure; the second axis represents residual variation not accounted for by water transparency. Species codes are given in Appendix B, Table B1. Be, Bélisle Brook; Br, Breckenridge Brook; G, Gatineau River; BG, Blanche à Gatineau River; PBG, Petite Blanche à Gatineau River; BT, Blanche à Thurso River; PBT, Petite Blanche à Thurso River; PN, Petite Nation River; K, Kinonge River; PK, Petite Kinonge River; R, Rouge River; Secchi, water transparency.



tained by the forward selection procedure ($P = 0.003$) (Fig. 4*a*). Water transparency explained 12.9% of the total variation (Fig. 3) (i.e., 33.3% of the variation among tributaries). Species associated with water transparency included emerald shiner, spottin shiner, fallfish, bluegill, rock bass, yellow perch, and logperch (*Percina caprodes*) (positive associations); and trout-perch, black crappie, central mudminnow, and brown bullhead (negative associations). Because in this analysis all within-tributary variation has been extracted by the covariates, in the ordination plot for sites (Fig. 4*b*), all sites within a tributary are located at the centroid specific to that tributary. Therefore, the scatter along axis 1 reflects solely variation among tributaries (axis 2 reflects only residual variation because there is a single environmental predictor in this analysis).

In the partial ordination analysis focusing on variation within tributaries (**T** as covariates), two environmental variables were selected by the forward selection procedure: macrophyte cover ($P = 0.002$) and river width ($P = 0.002$) (Fig. 5*a*). Macrophyte cover and river width jointly explained 5.1% of the total variation (Fig. 3) (i.e., 8.3% of the variation within tributaries). The first axis was associated with a gradient in macrophyte cover, whereas the second axis was associated with variation in river width. Both macrophyte cover and river width declined longitudinally as a function of distance to the Ottawa River (Fig. 6; mixed regression $P < 0.001$ for both relationships); however, distance to the Ottawa River itself was not significantly related to assemblage structure when it was included as a potential predictor in the ordination analyses. Neither regression required random effects for the slope, indicating that the effect of distance was similar across tributaries. Species associated with macrophyte cover included bluegill, pumpkinseed, yellow perch, banded killifish, and golden shiner (*Notemigonus crysoleucas*) (positive associations); and bluntnose minnow, smallmouth bass (*Micropterus dolomieu*), logperch, and johnny darter (negative associations). The species most strongly associated with river width were longnose dace

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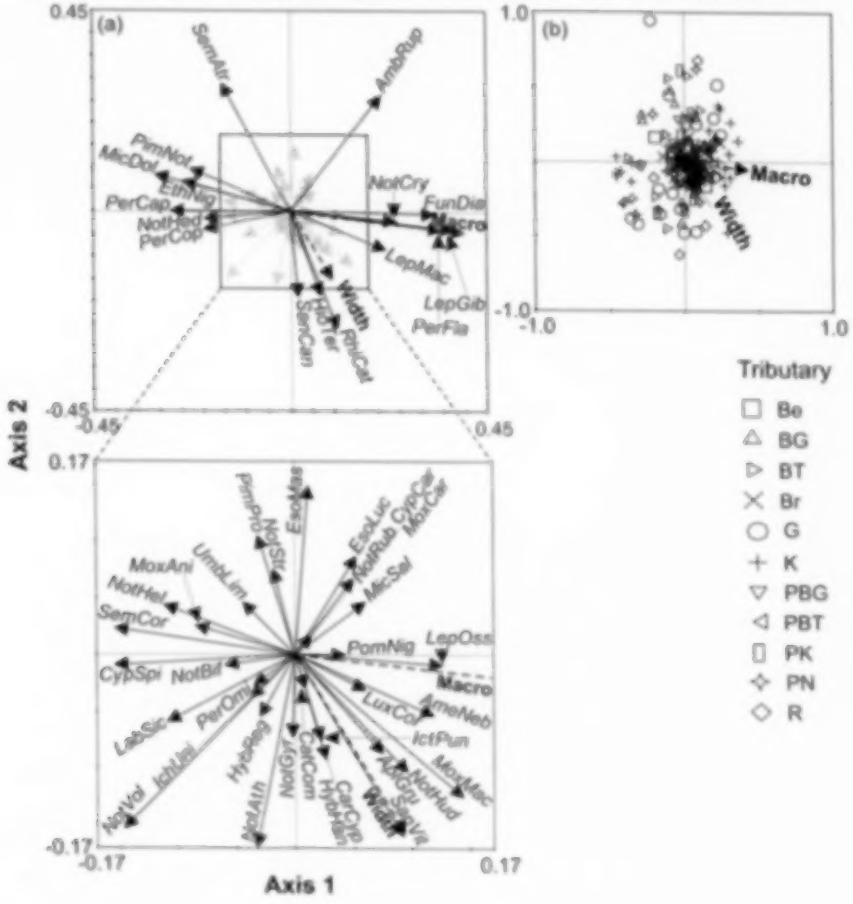
Fig. 5. (*a*) Species and (*b*) sites ordination plots for the partial redundancy analysis (RDA) focusing on within-tributary variation. The two ordination axes (axis 1: $P = 0.002$; axis 2: $P = 0.009$) jointly account for 8.3% of the within-tributary variation in assemblage structure. Species codes are given in Appendix B, Table B1. Be, Bélisle Brook; Br, Breckenridge Brook; G, Gatineau River; BG, Blanche à Gatineau River; PBG, Petite Blanche à Gatineau River; BT, Blanche à Thurso River; PBT, Petite Blanche à Thurso River; PN, Petite Nation River; K, Kinonge River; PK, Petite Kinonge River; R, Rouge River; Macro, macrophyte cover; Width, river width.



(*Rhinichthys cataractae*) (positive association) and creek chub (*Semotilus atromaculatus*) (negative association). Because in this analysis all among-tributary variation has been extracted by the covariates, in the ordination plot for sites (Fig. 5*b*), all tributaries have a common centroid at the origin. Therefore, the scatter reflects solely variation within tributaries, about the common centroid.

Marked differences between the full analysis and the partial analyses were found for some species responses. For example, rock bass showed an association with water transparency and river width in the full analysis (Fig. 2*a*), but the association with river width was no longer visible in the partial analysis focusing on within-tributary variation (Fig. 5*a*). However, the relationship with water transparency was still detectable in the partial analysis focusing on among-tributary variation (Fig. 4*a*).

The intraclass correlations showed that most of the variation in water transparency (ICC = 0.91) and river width (ICC = 0.85) was among tributaries (Table 2). In contrast, macrophyte cover was more variable within than among tributaries (ICC = 0.27). The mixed regression analyses indicated that PectHeight, DorsDist, Barbels, and Latstripe were related significantly to water transparency; PectHeight, DorsDist, and SwimF to macrophyte cover; and PectHeight and DorsDist to river width (Table 3). None of these models required random effects for the slope. Each of the trait–environment relationships was therefore characterized by a fixed slope common to all tributaries, indicating that environmental effects were similar across tributaries.

Discussion

Among-tributary variation

In the present study, 38.7% of the total variation was among tributaries, similar to a study of fish assemblages in three tributaries of the Elbe River, Czech Republic, which also found less variation among (22.5%) than within tributaries (Humpl and Pivnička 2006). Water transparency singly

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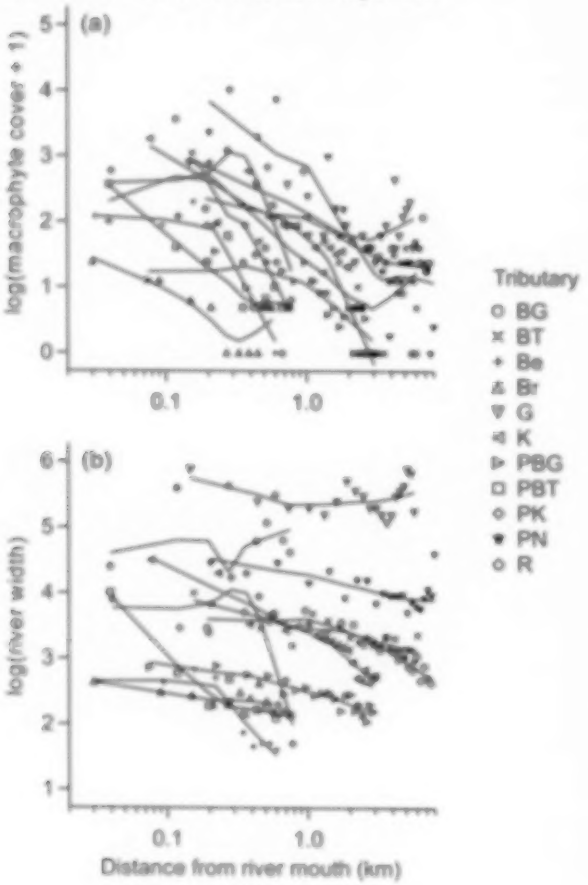
Fig. 6. Longitudinal change in (*a*) macrophyte cover (m; ln-transformed) and (*b*) river width (m; ln-transformed) for 170 sites distributed among the 11 tributaries of the Ottawa River included in this study. Be, Bélisle Brook; Br, Breckenridge Brook; G, Gatineau River; BG, Blanche à Gatineau River; PBG, Petite Blanche à Gatineau River; BT, Blanche à Thurso River; PBT, Petite Blanche à Thurso River; PN, Petite Nation River; K, Kinonge River; PK, Petite Kinonge River; R, Rouge River. The *x* axis represents distance from the mouth of the tributary (km; ln-transformed). Curves are LOWESS regressions.



accounted for a substantial fraction of the variation in assemblage structure among tributaries. Foraging performance in freshwater fish can depend critically on the match between sensory capacities and the optical environment. Water transparency has been shown to influence catchability of prey by visual foragers, such as bluegill (Luecke et al. 1990) and emerald shiner (Bonner and Wilde 2000). In floodplain lakes of the Orinoco River, Venezuela, fish with sensory adaptations to low light (e.g., well-developed lateral line system; chemical, tactile, or electric sensors) are dominant in turbid lakes, whereas fish relying on vision predominate in clear lakes (Rodríguez and Lewis 1997). The occurrence of sicklefin chub (*Macrhybopsis meeki*), which has elaborate sensory adaptations that promote foraging in turbid waters, has been shown to increase with turbidity along a longitudinal gradient in the Missouri River (Dieterman and Galat 2004).

The ecomorphological analyses pointed to links between water transparency and specific traits. The occurrence of barbels, which harbour sensitive tactile and chemical sensors, was negatively related to water transparency. This result reflects the association of brown bullhead, channel catfish (*Ictalurus punctatus*), and tadpole madtom (*Noturus gyrinus*) with turbid waters. Furthermore, the occurrence of high pectoral fins and a forward insertion of the dorsal fin, characteristic of species such as bluegill, yellow perch, golden shiner, and banded killifish, was greater in more transparent tributaries. These fins are related to maneuvering (Gibb et al. 1994; Standen and Lauder 2005), which suggests that species adept at maneuvering may rely primarily on vision to orient and forage. Eye diameter was not significantly related to water transparency in our study, contrary to previous work showing increased abundance of small-eyed species along a turbidity gradient in the upper Brazos River, Texas (Ostrand and Wilde 2002). Responses to low visibility in our study system may thus involve primarily nonvisual sensory adaptations. Alternatively, historical or phylogenetic constraints may limit the capacity for adaptive change in eye diameter.

In addition to modifying foraging behaviour, water transparency can mediate predator–prey relationships. In floodplain lakes, piscivores can shape assemblage structure by culling the most vulnerable prey species. Prey vulnerability is in turn strongly related to water transparency and to the sensory and foraging capabilities of individual species (Rodríguez and Lewis 1997). For small bluegill, a moderate increase in turbidity can reduce the risk of predation by largemouth bass in open water (Miner and Stein 1996). The ecomorphological analyses revealed a positive relationship between water transparency and the occurrence of a lateral stripe. Lateral stripes are commonly found in species that shoal and may contribute to coordinate shoal movements and confound visual predators (Krause and Ruxton 2002). Lateral stripes must lose effectiveness in these roles as visibility deteriorates, consistent with the finding that species having a lateral stripe (e.g., most *Notropis* species) were found in the most transparent tributaries.

Variation in water transparency among tributaries in this study system reflects predictable differences in sediment loading from the alluvial plain. Colonization of tributaries by species from the mainstem may not be random, but may instead reflect the sensory ecology of potential colonizers. If so, this would complement the work of Osborne and Wiley (1992), underscoring the importance of colonization processes from the mainstem to the tributaries in regulating local fish assemblages. Variation in water transparency was mostly among tributaries (ICC = 0.91), indicating that colonizers from the mainstem may be able to assess the transparency of a tributary from the confluence without exploring the tributary. Because of the importance of the optical environment to many fish species, differences in water transparency among tributaries, regardless of their origin, may contribute to structuring fish assemblages similarly in other riverine systems as well.

Within-tributary variation

Within-tributary variation in fish assemblage structure

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Table 3. Intercept (*a*), slope (*b*), and *P* value for the linear mixed regressions relating ecomorphological traits to the environmental variables retained in the ordination analyses.

	Environmental variable								
	Water transparency			Macrophyte cover			River width		
Trait	*a*	*b*	*P*	*a*	*b*	*P*	*a*	*b*	*P*
Eyediam	0.051	–0.001	0.155	0.047	0.001	0.302	0.048	–0.000	0.931
Pectlength	0.167	–0.001	0.833	0.155	0.006	0.032	0.142	0.007	0.116*
Pectheight	0.188	0.007	**0.004**	0.209	0.005	**0.001**	0.192	0.007	**<0.001**
Dorsdist	0.414	–0.009	**0.018**	0.385	–0.006	**0.019**	0.402	–0.007	**0.027**
Mouth	1.030	–0.005	0.534	0.991	0.018	0.077	0.921	0.032	0.128*
Barbels	0.310	–0.053	**0.002**	0.067	0.026	0.152	–0.039	0.054	0.244
Patterns	1.322	–0.040	0.347	1.071	0.062	0.018	1.219	–0.018	0.606
Silver	0.272	0.021	0.705	0.417	–0.042	0.123	0.190	0.050	0.250
Latstripe	0.024	0.085	**0.001**	0.430	–0.041	0.087	0.221	0.046	0.067
ShapeF	1.241	0.025	0.441	1.423	–0.066	0.035	1.474	–0.058	0.253
SwimF	–0.753	0.006	0.614	–0.770	0.031	**<0.001**	–0.763	0.010	0.316

Note: The three environmental variables were ln-transformed. Bold data indicate significant relationships after adjustment by the false discovery rate procedure. With two exceptions (see footnote), all models have a random intercept and a fixed slope.

*Random intercept and slope.

was influenced mostly by longitudinal variation in macrophyte cover and river width. Many studies have shown that both fish abundance and species richness increase with macrophyte cover (e.g., Grenouillet et al. 2002; Snickars et al. 2006), presumably because vegetated habitats provide increased food availability (Grenouillet et al. 2002) and refuge against predators (Sass et al. 2006). The ecomorphological analyses indicated that macrophyte cover was positively related to the occurrence of high pectoral fins and a forward insertion of the dorsal fin, which confer maneuverability as described earlier. Maneuvering abilities related to pectoral fin size or position may confer competitive advantages when macrophyte cover is abundant. Bluegills with longer pectoral fins search for food more slowly and spend more time in vegetated habitat compared with bluegills with shorter pectoral fins, which spend more time in open-water habitat (Ehlinger 1990). Similarly, European perch (*Perca fluviatilis*) has higher foraging efficiency than European ruddi (*Scardinius erythrophthalmus*) and European roach (*Rutilus rutilus*) in dense macrophyte beds, presumably because of the forward positioning of the pectoral fins in the perch (Winfield 1986). The ecomorphological analyses also indicated a positive relationship between macrophyte cover and swimming factor. Fish with a low swimming factor, such as thunniforms, are capable of strong, sustained swimming (Poff and Allan 1995). However, cruising and maneuvering abilities are usually negatively correlated (Lauder and Drucker 2004), suggesting that the negative relationship between swimming factor and macrophyte cover reflects a trade-off favouring maneuvering over cruising ability in vegetated, structurally complex environments. Macrophyte cover was linked with traits related to maneuvering but not to traits related to predator avoidance (e.g., shape factor, protective colouration), suggesting that vegetated habitat influenced community structure by allowing for efficient exploitation of abundant food resources rather than by providing refuge from predators. In contrast with transparency, variation in macrophyte cover was mostly within tributaries (ICC = 0.27), indicating that potential colonizers from the mainstem may have to enter and explore a tributary to assess macrophyte cover.

The second environmental variable associated with the within-tributary variation was river width. Although river width was positively related to macrophyte cover in the present study (median Spearman correlation = 0.62 for the 11 tributaries), the apparent effect of river width on assemblage structure is independent of macrophyte cover, which was partialled out in the ordination analysis. High pectoral fins and a forward insertion of the dorsal fin were also positively related to river width. This result agrees with the finding that maneuverers were more abundant in larger rivers in a study of 429 fish species from the Mississippi River basin (Goldstein and Meador 2004). The positive relationship between river width and maneuverability may be related to increased availability of slower flow in wider river stretches.

In conclusion, the partial analyses emphasizing among- and within-tributary components of variation in assemblage structure provided a more complete interpretation than the full analysis by showing that two distinct sets of environmental variables were associated with variation among and within tributaries. Because the full ordination combines and simultaneously represents species–environment relationships among and within tributaries, a trade-off in this representation is inevitable whenever the among- and within-tributary relationships differ. The partial ordinations also indicated that although variation in assemblage structure was greater within than among tributaries, the proportion of variation explained by environmental variables was greater among than within tributaries. These results suggest that differences in habitat features among tributaries may account for a substantial fraction of the predictable variation in assemblage structure at the watershed scale, an aspect not emphasized in previous studies of riverine fish assemblages. Our results also showed that the environmental variables most strongly associated with assemblage structure were significantly related to ecomorphological traits generally associated with foraging efficiency.

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Appendices A and B appear on the following pages.

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Appendix A

Table A1. Example of effects coding for model terms in a hypothetical study of 21 sites distributed among five tributaries: three

			Tributaries (rank = 4)				Sites (rank = 5)				
Tributary	Site	$\boldsymbol{C}_1$	$\mathbf{T}_1$	$\mathbf{T}_2$	$\mathbf{T}_3$	$\mathbf{T}_4$	$\mathbf{S}_1$	$\mathbf{S}_2$	$\mathbf{S}_3$	$\mathbf{S}_4$	$\mathbf{S}_5$
1	1	1	−1	−1	−1	−1	−1	−1	0	0	0
	2	1	−1	−1	−1	−1	1	0	0	0	0
	3	1	−1	−1	−1	−1	0	1	0	0	0
2	1	1	1	0	0	0	−1	−1	0	0	0
	2	1	1	0	0	0	1	0	0	0	0
	3	1	1	0	0	0	0	1	0	0	0
3	1	1	0	1	0	0	−1	−1	0	0	0
	2	1	0	1	0	0	1	0	0	0	0
	3	1	0	1	0	0	0	1	0	0	0
4	1	1	0	0	1	0	−1	−1	−1	−1	−1
	2	1	0	0	1	0	1	0	0	0	0
	3	1	0	0	1	0	0	1	0	0	0
	4	1	0	0	1	0	0	0	1	0	0
	5	1	0	0	1	0	0	0	0	1	0
	6	1	0	0	1	0	0	0	0	0	1
5	1	1	0	0	0	1	−1	−1	−1	−1	−1
	2	1	0	0	0	1	1	0	0	0	0
	3	1	0	0	0	1	0	1	0	0	0
	4	1	0	0	0	1	0	0	1	0	0
	5	1	0	0	0	1	0	0	0	1	0
	6	1	0	0	0	1	0	0	0	0	1

Note: The table includes coefficients for a constant term, $\boldsymbol{C}$ (a column vector of ones), which is included in all models, and three matrices of variables, taries and sites ($\mathbf{TS}$). All of the matrices have full rank. Design matrices for different models can be constructed as combinations of the constant vector $\boldsymbol{C}$ among tributaries, includes the five columns associated with terms $\boldsymbol{C}$ and $\mathbf{T}$. The design matrix for model $\boldsymbol{C} + \mathbf{T} + \mathbf{S} + \mathbf{TS}$ includes all 21 columns, has full

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tributaries with three sites each and two tributaries with six sites each.

Tributaries × Sites interaction (rank = 11)										
TS_1	TS_2	TS_3	TS_4	TS_5	TS_6	TS_7	TS_8	TS_9	TS_{10}	TS_{11}
1	1	1	1	1	1	1	1	0	0	0
−1	−1	−1	−1	0	0	0	0	0	0	0
0	0	0	0	−1	−1	−1	−1	0	0	0
−1	0	0	0	−1	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0
0	0	0	0	1	0	0	0	0	0	0
0	−1	0	0	0	−1	0	0	0	0	0
0	1	0	0	0	0	0	0	0	0	0
0	0	0	0	0	1	0	0	0	0	0
0	0	−1	0	0	0	−1	0	−1	−1	−1
0	0	1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	1	0	0	0	0
0	0	0	0	0	0	0	0	1	0	0
0	0	0	0	0	0	0	0	0	1	0
0	0	0	0	0	0	0	0	0	0	1
0	0	0	−1	0	0	0	−1	0	0	0
0	0	0	1	0	0	0	0	0	0	0
0	0	0	0	0	0	0	1	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0

constructed as effects coding for tributaries (**T**), sites within tributaries (**S**), and the interaction between tributwith the **T**, **S**, and **TS** matrices. For example, the design matrix for model $C + T$, which accounts for variation rank, and accounts for all the variation in assemblage structure because its rank equals the number of sites.

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Appendix B

Table B1. Mean number per site and percentage of the total number of fish for 49 fish species in the 11 tributaries of the Ottawa River included in this study.

Family	Scientific name	Common name	Code	Mean	Percentage
Atherinopsidae	*Labidesthes sicculus*	Brook silverside	LabSic	0.03	0.03
Catostomidae	*Carpiodes cyprinus*	Quillback	CarCyp	0.05	0.04
	Catostomus commersonii	White sucker	CatCom	0.61	0.53
	Moxostoma anisurum	Silver redhorse	MoxAni	0.06	0.05
	Moxostoma carinatum	River redhorse	MoxCar	0.01	0.01
	Moxostoma macrolepidotum	Shorthead redhorse	MoxMac	0.06	0.05
Cyprinidae	*Cyprinus carpio*	Common carp	Cypcar	0.04	0.03
	Hybognathus hankinsoni	Brassy minnow	HybHan	0.01	0.01
	Hybognathus nuchalis	Mississippi silvery minnow	HybNuc	3.16	2.76
	Luxilus cornutus	Common shiner	LuxCor	1.14	0.99
	Notemigonus crysoleucas	Golden shiner	NotCry	2.40	2.09
	Notropis atherinoides	Emerald shiner	NotAth	8.36	7.28
	Notropis bifrenatus	Bridled shiner	NotBif	0.01	0.01
	Notropis heterodon	Blackchin shiner	NotHed	0.02	0.02
	Notropis heterolepis	Blacknose shiner	NotHel	0.04	0.04
	Notropis hudsonius	Spottail shiner	NotHud	1.26	1.10
	Notropis rubellus	Rosyface shiner	NotRub	0.02	0.02
	Cyprinella spiloptera	Spotfin shiner	CypSpi	0.52	0.46
	Notropis stramineus	Sand shiner	NotStr	0.04	0.03
	Notropis volucellus	Mimic shiner	NotVol	2.98	2.60
	Pimephales notatus	Bluntnose minnow	PimNot	1.39	1.21
	Pimephales promelas	Fathead minnow	PimPro	0.02	0.02
	Rhinichthys cataractae	Longnose dace	RhiCat	0.01	0.01
	Semotilus atromaculatus	Creek chub	SemAtr	0.01	0.01
	Semotilus corporalis	Fallfish	SemCor	2.20	1.92
Esocidae	*Esox lucius*	Northern pike	EsoLuc	0.35	0.30
	Esox masquinongy	Muskellunge	EsoMas	0.16	0.14
Fundulidae	*Fundulus diaphanus*	Banded killifish	FunDia	0.12	0.10
Umbridae	*Umbra limi*	Central mudminnow	UmbLim	1.04	0.90
Lepisosteidae	*Lepisosteus osseus*	Longnose gar	LepOss	0.05	0.04
Hiodontidae	*Hiodon tergisus*	Mooneye	HioTer	0.15	0.13
Centrarchidae	*Ambloplites rupestris*	Rock bass	AmbRup	8.36	7.28
	Lepomis gibbosus	Pumpkinseed	LepGib	17.40	15.20
	Lepomis macrochirus	Bluegill	LepMac	5.21	4.53
	Micropterus dolomieu	Smallmouth bass	MicDol	0.49	0.43
	Micropterus salmoides	Largemouth bass	MicSal	5.99	5.22
	Pomoxis nigromaculatus	Black crappie	PomNig	23.91	20.82
Percidae	*Etheostoma nigrum*	Johnny darter	EthNig	2.29	1.99
	Percina caprodes	Logperch	PerCap	2.19	1.91
	Percina copelandi	Channel darter	PerCop	0.12	0.11
	Perca flavescens	Yellow perch	PerFla	17.91	15.60
	Sander canadensis	Sauger	SanCan	0.18	0.16
	Sander vitreus	Walleye	SanVit	0.15	0.13
Sciaenidae	*Aplodinotus grunniens*	Freshwater drum	AplGru	0.04	0.03
Percopsidae	*Percopsis omiscomaycus*	Trout-perch	PerOmi	2.01	1.75
Petromyzontidae	*Ichthyomyzon unicuspis*	Silver lamprey	IchUni	0.01	0.01
Ictaluridae	*Ameiurus nebulosus*	Brown bullhead	AmeNeb	2.23	1.94
	Ictalurus punctatus	Channel catfish	IctPun	0.01	0.01
	Noturus gyrinus	Tadpole madtom	NotGyr	0.03	0.03

Note: A total of 19 518 fish were captured.

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Can bottom trawling disturbance increase food production for a commercial fish species?

Jan Geert Hiddink, Adriaan D. Rijnsdorp, and Gerjan Piet

Abstract: Fishery closures and marine protected areas are increasingly being used as tools to achieve sustainable fisheries. The "plaice box", a gear restriction area in the North Sea that was established to reduce the bycatch of undersized plaice (*Pleuronectes platessa*), is considered ineffective because there has been a shift in the distribution of juvenile plaice to the waters that remained open to bottom trawlers. Here we examine the hypothesis that bottom trawling benefits the small benthic invertebrates that form the food source for plaice and that the plaice box had a negative impact on food production for plaice. A size-based model of benthic communities indicates that the production of prey was low without trawling and maximal in areas that are trawled once to twice a year. Therefore, bottom disturbance may improve the feeding conditions for species that feed on small invertebrates. As plaice aggregate at the locations with the highest benthic biomass, this may explain the observed redistribution to areas outside the plaice box. We conclude that the plaice box may not have been the most appropriate measure to protect plaice from discarding and that the species' ecology should be considered when choosing the most appropriate management measure to achieve an objective.

Résumé : Les fermetures de la pêche et les zones de protection marine servent de plus en plus d'outils pour obtenir des pêches durables. Le « cantonnement à plies », une zone de restriction des engins de pêche dans la mer du Nord établie pour réduire la capture accessoire de plies (*Pleuronectes platessa*) de taille inférieure à la limite, est considérée inefficace car il y a eu un déplacement dans la répartition des plies vers les eaux qui restent disponibles aux navires de chalutage de fond. Nous examinons ici l'hypothèse selon laquelle le chalutage de fond favorise les petits invertébrés benthiques qui constituent la source de nourriture des plies et que le cantonnement à plies a un impact négatif sur la production de nourriture des plies. Un modèle des communautés benthiques basé sur la taille indique que la production de proies est basse en l'absence de chalutage et qu'elle est maximale dans les zones où il y a du chalutage une ou deux fois par an. C'est pourquoi, la perturbation du fond peut améliorer les conditions alimentaires pour les espèces qui se nourrissent de petits invertébrés. Comme les plies se rassemblent dans les sites de biomasse benthique maximale, cela peut expliquer le redéploiement des plies vers les zones extérieures au cantonnement à plies. Nous concluons que le cantonnement à plies ne s'est peut-être pas avéré être la méthode la plus appropriée pour protéger les plies du rejet à la mer; il faut donc tenir compte de l'écologie de l'espèce lors du choix de la mesure de gestion la plus appropriée pour réaliser un objectif.

[Traduit par la Rédaction]

Introduction

Marine protected areas (MPAs) are areas where the environment is protected in some way and include marine reserves, no-take zones, and fishery closures (Kelleher 1999). MPAs are increasingly being used as a tool for achieving sustainable fisheries. There exists an extensive literature on the contributions of MPAs to achieve conservation goals (meta-analysis in Halpern 2003). However, the fishery benefits of MPAs are ambiguous (Willis et al. 2003; Gårdmark et al. 2006), and the negative effects of displacement of fishing effort from the closed areas to areas that remain open may be larger than the positive effects in the closed areas (Dinmore et al. 2003; Hiddink et al. 2006*a*).

The "plaice box" is a 38 000 km^2 gear restriction area (a type of MPA) in the southeastern North Sea that was established to reduce the bycatch of undersized plaice (*Pleuronectes platessa*) in heavily trawled coastal nursery grounds. Large mature plaice are distributed more widely and further offshore than juveniles (Rijnsdorp and van Leeuwen 1996). The plaice box was closed to trawlers with an engine power >300 hp (1 hp = 746 W) for part of the year in 1989 and for the whole year since 1995. There are no spatial restrictions on fishing by trawlers with an engine power <300 hp and fishing effort by these vessels in the plaice box has increased since 1989. Nevertheless, total trawling effort in the plaice box was reduced by more than 90%, and most of this effort was redistributed into adjacent areas (Pastoors et al. 2000). At the time of closure, it was expected that this closure would result in an increase in plaice stocks of 25%–35% (Grift et al. 2004), but instead plaice spawning stock biomass has declined strongly (Pastoors et al. 2000). There also seems to have been a shift in the spatial distribution of juvenile plaice to the deeper offshore waters that have remained open to all trawlers (Van Keeken et al. 2007). Therefore, the plaice box is currently

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less effective than anticipated in reducing the capture and discarding of juvenile plaice (Grift et al. 2004). Several explanations for the decrease in abundance of juvenile plaice in the plaice box have been put forward, such as decreasing eutrophication, climate change, and a decrease in intraspecific competition in offshore areas and an increase in predation risk in shallow waters (Van Keeken et al. 2007). Another explanation, which is endorsed by fishermen, is that bottom trawling "farms the sea" (Rijnsdorp and Van Beek 1991). The idea behind this is that ploughing the seabed with heavy beam trawls increases the production of the small benthos that flatfish feed on by removing the large fauna that small benthos compete with over food and space. Benthic production is the amount of biomass that is generated by benthic invertebrates in a year and therefore determines the amount of food that becomes available for higher trophic levels. According to this hypothesis, the reduction of fishing effort in the plaice box has led to a decrease in bottom disturbance, an increase in the abundance of large-bodied invertebrates, and a concordant reduction in the production of flatfish food. Aggregation of juvenile plaice in the habitats with the highest production of food will then result in a redistribution to the heavily trawled areas outside the plaice box (Rijnsdorp and Vingerhoed 2001; Grift et al. 2004; Van Keeken et al. 2007). Evidence for aggregation at the scales we examine in this paper has not been studied for plaice, but has been found for Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) in the North Sea (Blanchard et al. 2005; Hiddink et al. 2005).

Previous studies have shown that bottom trawling has a negative effect on the total production of benthic invertebrate communities (Jennings et al. 2001; Hermsen et al. 2003; Hiddink et al. 2006*b*). The abundance of large invertebrates is strongly reduced by chronic trawling disturbance because of their slow life history (Jennings et al. 2001), and models suggest that reduced competitive interactions between these large invertebrates and small invertebrates may lead to an increase in the abundance of the latter (Duplisea et al. 2002). Plaice diets consist primarily of these small infaunal invertebrates, in particular polychaetes and to a lesser extent bivalves (Wyche and Shackley 1986; Piet et al. 1998; Rijnsdorp and Vingerhoed 2001). Therefore, the production of plaice food may have decreased in the plaice box when trawling effort was reduced.

In the current study, we explore the hypothesis that a reduction in trawling disturbance in the plaice box had a negative impact on the production of food for plaice by using an established size-based model of invertebrate communities (Hiddink et al. 2006*b*). Here, we assess the size range of food items taken by plaice from stomach content analysis. The size-based model is then used to predict the production of the prey of plaice inside and outside the plaice box. Most studies to date that have considered MPAs as fisheries management tools have examined the effects of MPAs on fish populations through population dynamics and dispersal. The current study is one of the first to consider the effect of an MPA on the trophic ecology of a commercial fish species. Understanding the impact of bottom trawl fisheries on the production of their food for commercial fish is of vital importance when designing and evaluating the utility of MPAs.

Materials and methods

In this paper, we estimate food availability for plaice using a model, rather than by examining empirical data on plaice diet, for several reasons. Direct comparison of the diet of plaice is unlikely to be productive when trying to assess differences in food availability between locations when fish show density-dependent habitat selection and distribute themselves over food resources according to the ideal free distribution (Fretwell and Lucas 1969; Shepherd and Litvak 2004). The ideal free distribution dictates that the fish are distributed in a way that the same amount of food is available for each fish. In this case, no differences in the diet between fish inside and outside the MPA would be expected, and a previous study has shown that no such differences exist (Rijnsdorp and Vingerhoed 2001). Currently, no large-scale data on the abundance of benthic invertebrates for the plaice box and surrounding area exists, and the little data that exists does not allow separation of benthic communities into size classes and is therefore unsuitable for our analysis. Ideally, we would manipulate trawling effort in experimental areas in and outside the plaice box and record the response of benthic communities to these manipulations. However, such large-scale manipulations of fishing effort are currently not within our reach. For these reasons, we tackle our question by combining general diet data with a model that predicts the impact of trawling on benthic communities. This approach can test the hypothesis that changes in benthic production explain changes in the distribution of juvenile plaice, but does not test or reject alternative hypotheses. It should therefore be seen as a starting point for future modelling and empirical studies on the indirect effects of trawling and MPAs.

Plaice stomach contents

Plaice were collected for stomach content analysis in the North Sea (NS) and the English Channel (EC) (Fig. 1). Plaice were assigned to 5 cm length classes. NS samples were collected in 1996 and used in a separate previous analysis (Rijnsdorp and Vingerhoed 2001). In short, stomachs of plaice were collected inside and outside the plaice box on several cruises from April to September 1996 using beam trawls. In total, 1337 stomachs containing food were sampled. More details of the sampling methods are given in Rijnsdorp and Vingerhoed (2001).

Plaice stomachs (n = 222) were collected from 11 stations in the EC in August 2002. Stomachs were preserved in 8% buffered formalin. The sampling protocol is described in more detail by Hinz et al. (2006). The abundance and wet weight (WW) of all dry-blotted prey was recorded and assigned as soft or hard. "Soft" are soft-bodied animals like polychaetes, and "hard" are animals with a hard exoskeleton like bivalves, echinoderms, and crustaceans. Unidentified remains were ignored in the analysis (EC: 5.2% by weight; NS: <1% by weight). The digestive state and the level of intactness of prey items were not recorded, and this will have caused an underestimate of the weight of prey (Hyslop 1980). All individual food items in the stomach were assigned to $\log_2$ weight (g WW) classes. The classes are reported as the lower end of the class interval.

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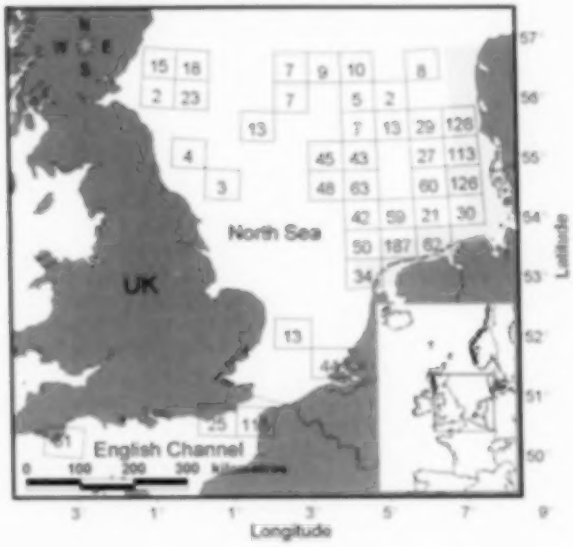
Fig. 1. The number of plaice (*Pleuronectes platessa*) stomachs sampled per ICES (International Council for the Exploration of the Sea) rectangle in the North Sea (data from Rijnsdorp and Vingerhoed 2001) and the English Channel. The extent of the plaice box is indicated by grey shading.



Plaice abundance – benthos abundance relationships

The abundance of plaice was related to the biomass of infaunal invertebrates using samples collected on nine stations in the EC in August 2002. At each station, three–five 0.1 m^2 Day grabs were taken and sieved over a 1 mm mesh. Animals were sorted to species and wet-weighed individually after blotting. The total biomass of invertebrates <0.5 g WW·m^{-2} was calculated. Plaice abundance was estimated in daylight hours using a 4 m beam trawl fitted with a chain matrix and an 82 mm diamond mesh cod end with a 40 mm square mesh liner. Four tows of 20 min duration were made at each site at speed of 4 kn (1 kn = 1.853 km·h^{-1}). Details of the sampling stations and methodology are given in Hinz et al. (2006) and Shucksmith et al. (2006).

Benthos model

We used a size-based model of the response of soft-sediment benthic communities to trawling disturbance that has been validated with extensive field data to examine the large-scale impact of trawling disturbance on benthic production. Details of the model are given in Duplisea et al. (2002) and Hiddink et al. (2006*b*). In summary, the model contains 32 state variables in two faunal groups (soft and hard macrofauna): 16 for soft (1.9–500 mg) and 16 for hard (50 – 60 000 mg). At the same size, soft have life-history characteristics that allow them to grow faster than hard. Duplisea et al. (2002) give the life-history parameters for soft and hard in all size classes. Soft and hard were assumed to be in competition over food or space (Wilson 1990). Growth of the population biomass in each body mass – organism type compartment is modelled by modifying Lotka–Volterra competition equations to give the population biomass flux for a compartment. The interaction between habitat type and trawling effects is modelled by including relationships between growth, mortality, and the environment in the model. Thus, sediment type affects trawling mortality, sediment erosion rates affect natural mortality, the effect of bed shear stress modifies population growth rate, and the chlorophyll *a* content of the sediment affects carrying capacity. The interaction of organisms with the environment is independent of body type and depends solely on their life-history parameters. As soft- and hard-bodied invertebrates have different life-history parameters in the model, the latter dominate stable habitats and the former prevail in the more dynamic and disturbed habitats. A critical assumption of the benthos model is that there is competition between different size classes of benthic invertebrates. This means that without disturbance large animals out-compete the smaller animals. The impact of plaice predation on benthic biomass was not considered. The model has been validated using benthic biomass and production estimates from 33 stations subject to a range of trawling frequencies in four areas in the NS. For a detailed description of the model, validation, and the environmental data sets that were used, see Hiddink et al. (2006*b*). The model was run to equilibrium in 1500 time steps of 30 days for each individual cell in the NS, assuming fishing effort was constant over this period.

Benthic production was modelled for 9 km^2 cells in the plaice box (n = 2461) and a reference area within 50 km of its borders (n = 2925). These areas are comparable, but not identical, in environmental conditions. Sediment composition was particularly divergent, with mud and muddy sand covering 34% of the area outside the plaice box and only 3% inside the plaice box. Average depth was 22 m inside the plaice box and 35 m in the adjacent area. Apart from trawling frequency, none of the environmental parameters showed a sudden change that coincided with the boundary of the plaice box. To the north (56 N) and east (8 E), the modelled area was limited by the availability of environmental data.

Trawling effort

Trawling frequency was calculated from European Community Satellite Vessel Monitoring System data of Dutch beam trawl vessels and raised to the total fishing effort of the Dutch fleet (for more details see Dinmore et al. 2003; Hiddink et al. 2006*b*). To distinguish fishing from other activities (e.g., steaming), this approach uses the speed of the vessel and knowledge on the range of speeds per gear type at which fishing is conducted (Piet et al. 2007). As this approach is not perfect and the results of this study are sensitive to spurious fishing registrations, notably in the plaice box, we added another filter and removed all registrations of the larger vessels (>300 hp) that are not allowed to fish in the box. These registrations were generally found on routes to and from fishing ports and were therefore considered unlikely to represent illegal fishing. Trawling frequency was expressed as the number of times each 9 km^2 grid cell was swept each year. Although there is interannual variation in fishing effort, we used the mean trawling frequencies for the period 2000–2004 and assumed these are representative for the most recent period. To check the sensitivity of our

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Table 1. The six most abundant (by weight) prey items in plaice *Pleuronectes platessa* stomachs from the English Channel and North Sea.

Species	Phylum	Fraction by weight
English Channel		
Lanice conchilega	Annelida	0.43
Ensis arcuatus	Bivalvia	0.07
Nephtys spp.	Annelida	0.05
Amphiura spp.	Echinodermata	0.04
Echinocardium cordatum	Echinodermata	0.03
Notomastus latericeus	Annelida	0.03
Total		0.64
North Sea		
Pectinaria spp.	Annelida	0.16
Nereis spp.	Annelida	0.14
Ensis spp.	Bivalvia	0.10
Spisula spp.	Bivalvia	0.08
Liocarcinus holsatus	Crustacea	0.06
Upogebia spp.	Crustacea	0.05
Total		0.59

conclusions to the trawling effort estimates, we corrected the Dutch trawling effort data to represent international (including The Netherlands) and historic trawling regimes (1990–1995) using International Council for the Exploration of the Sea (ICES) fishing effort data per ICES rectangle, assuming that the small-scale effort within the rectangles remained the same (Hiddink et al. 2006*a*). The Dutch fishing effort is therefore a subset of the international fishing effort. This resulted in four different effort estimates: Netherlands 2000–2004, International 2000–2004, Netherlands 1990–1995, and International 1990–1995. The years 1990–1995 are considered most representative of the period in which the distribution of juvenile plaice shifted to more offshore areas. The Dutch beam trawl fleet represented about 75% of the international trawling effort in the plaice box from 2000 to 2004 (Van Keeken et al. 2007).

Environmental data

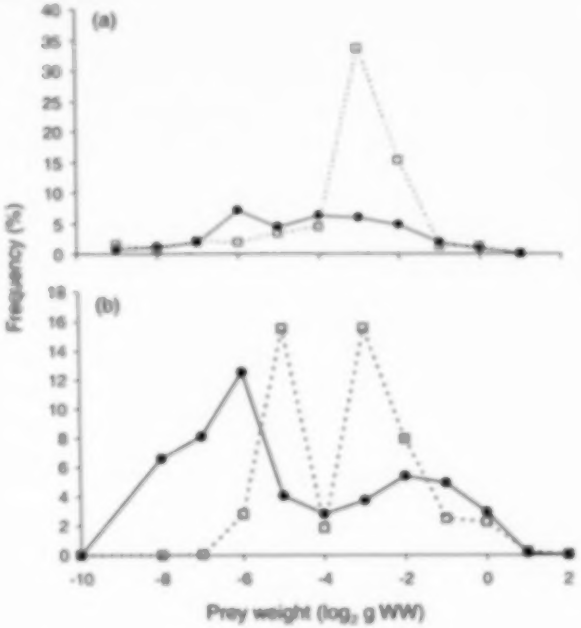
The sources of habitat data are described by Hiddink et al. (2006*b*). Additional seabed sediment composition data were obtained for the German Bight (Figge 1981) and for Danish waters (Bundesanstalt für Bodenforschung and Unesco 1970).

Results

Stomach contents

The most dominant prey of plaice in the EC and the NS were several species of infaunal polychaetes (Table 1). Very few prey over 2 g WW ($\log_2$ class 0) and below 4 mg WW ($\log_2$ class –8) were found in plaice stomachs (Fig. 2). In the EC, most prey were found in the soft –3 $\log_2$ weight class (125–250 mg WW). In the NS, the soft-bodied fauna showed a bimodal frequency distribution, with many prey items in the –5 (31–63 mg) and –3 (125–250 mg WW) $\log_2$ weight classes. This bimodal distribution was caused by the high abundance of the polychaete *Magelona* spp. in the –5 size class and by the high abundance of the polychaete *Pectinaria* spp. in the –3 size class. Most hard-bodied fauna in the stomachs weighed between 4 and 31 mg ($\log_2$ weight classes –8 to –6).

Fig. 2. Frequency distribution of prey in plaice (*Pleuronectes platessa*) stomach content per $\log_2$ weight class (g wet weight, WW): (*a*) English Channel, (*b*) North Sea. Circles and solid lines represent hard-bodied infauna; squares and broken lines indicate soft-bodied infauna.



Small plaice ingested smaller prey than large plaice; the modal size of prey in the stomach was significantly positively related to plaice length class, although only a small fraction of the observed variation in prey weight was explained by plaice length (R^2 = 0.20; area: $F_{[1,1179]}$ = 158, p < 0.001; size class: $F_{[1,1179]}$ = 150, p < 0.001). At the same size, plaice ingested smaller prey in the EC than in the NS. Juvenile plaice (up to 25 cm, 1–2 years old) primarily ingested prey in the –4 to –3 $\log_2$ size class (62.5–250 mg). In both the NS and the EC, soft-bodied invertebrates dominated the stomach contents of 15–25 cm plaice by weight (EC = 64%, NS = 60%).

Relation between plaice abundance and benthic biomass

In the EC, plaice were more abundant on stations with a high biomass of small benthic invertebrates (Fig. 3, R^2 = 0.50, $F_{[1,8]}$ = 8.11, p = 0.021).

Model predictions

The modelled response of production to trawling was very similar inside and outside the plaice box (Fig. 4). Total production of the whole benthic community was highest without trawling, but production of animals <0.5 g peaked at trawling frequencies of 0.25 year^{-1}. The production of small (<500 mg) soft- and hard-bodied invertebrates reacted dif-

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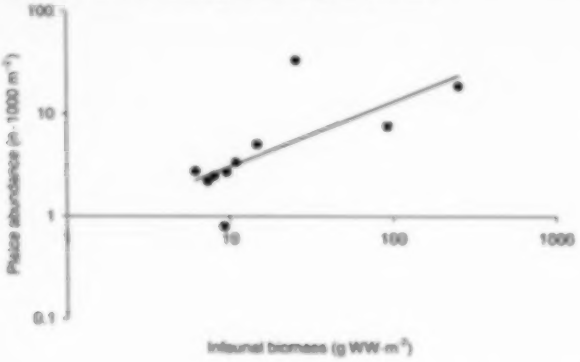
Fig. 3. Relationship between plaice (*Pleuronectes platessa*) abundance and food availability in the English Channel.



ferently to trawling disturbance (Fig. 4). The production of hard-bodied invertebrates decreased strongly with trawling to virtually nothing at a trawling frequency of 0.5 year^{-1}. Production of soft-bodied invertebrates was low at very low trawling frequencies, but increased to a maximum at trawling frequencies of 0.5–1 year^{-1} and decreased slowly with further increasing trawling frequencies.

Trawling effort was much higher outside than inside the plaice box and was particularly intense in the southeastern NS outside the boundary (Fig. 4*a* and Fig. 5*a*). Typical international trawling frequencies in the plaice box (<0.5 year^{-1}) resulted in a high production of hard-bodied invertebrates and a low production of soft–bodied invertebrates (Fig. 4). International trawling frequencies in the reference area around the plaice box (>2 year^{-1}) resulted in a maximal production of soft-bodied invertebrates, but a low production of hard-bodied invertebrates.

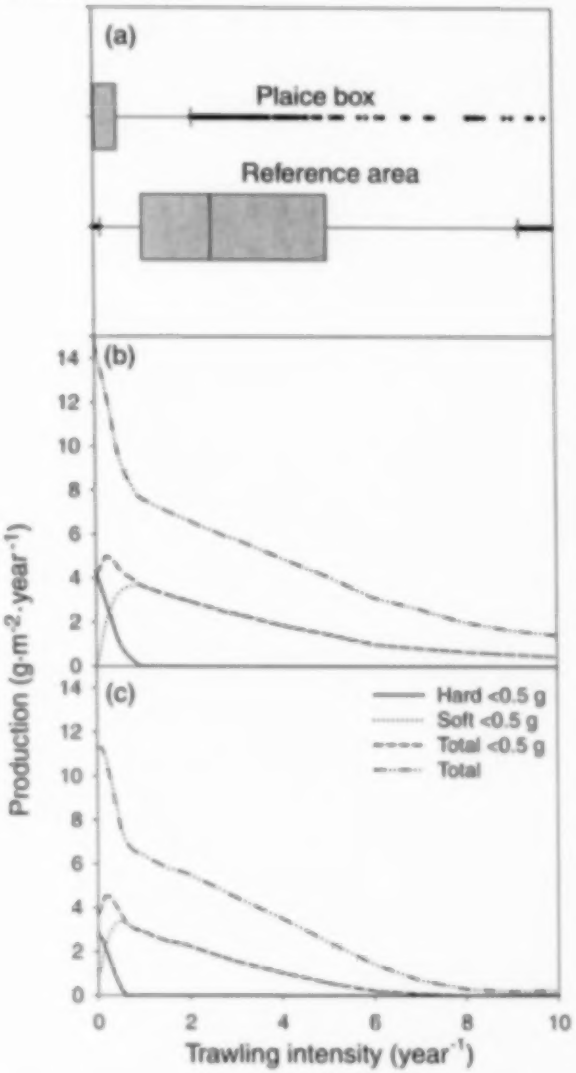
The modelled production of small, soft-bodied invertebrates (<500 mg) showed a gradient that coincided with the boundary of the plaice box and was 1.5 to 2 times lower (depending on the effort estimate used) in the plaice box than in the reference area (Fig. 5*b*, Table 2). Total benthic production (all size classes and both soft- and hard-bodied invertebrates) was much higher than the production of small, soft-bodied invertebrates and was 1.5 to 2 times higher in the plaice box than in the reference area (Fig. 5*c*).

The effect of different estimates of fishing effort on the relative benthic production inside and outside the plaice box was relatively small (Table 2). For all four effort estimates, total benthic production was higher in the plaice box, while production of small, soft-bodied benthic invertebrates was higher outside the plaice box.

Discussion

The plaice box has successfully reduced discarding of juvenile plaice within this MPA (Grift et al. 2004). Our analysis provides support for the hypothesis that the reduction of fishing effort in the plaice box has led to a decrease in bottom disturbance and may lead to a concordant reduction of the production of flatfish food in the plaice box. Decreasing trawling disturbance favours large, inedible (for plaice), benthic invertebrates over small, edible invertebrates. As a result of this indirect effect, production of small, soft-bodied

Fig. 4. (*a*) Box and whisker plot of trawling frequency (international effort 2000–2004) in the plaice box and the reference area. The boundaries of the boxes indicate the 25th and the 75th percentiles, the line within the box marks the median, and the whiskers indicate the 90th and 10th percentiles. Outlying points are plotted as individual points; outliers over 10 are not shown. The median for the plaice box is 0. (*b*) The average modelled relationship between trawling frequency and benthic production in the plaice box. (*c*) The average modelled relationship between trawling frequency and benthic production in the reference area in the southeastern North Sea.



invertebrates (and food availability for plaice) is predicted to be higher outside the plaice box. These model results thus provide support for the idea that disturbing the seabed with bottom trawls may increase food production for fish species that feed on small invertebrates. These predictions have not been corroborated by empirical results for the plaice box area (although the model has been validated in the southern

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Fig. 5. Trawling frequency and modelled benthic invertebrate production in and around the plaice box: (*a*) international trawling frequency 2000–2004; (*b*) production of small, soft-bodied invertebrates (<500 mg); (*c*) production of the whole benthic community.

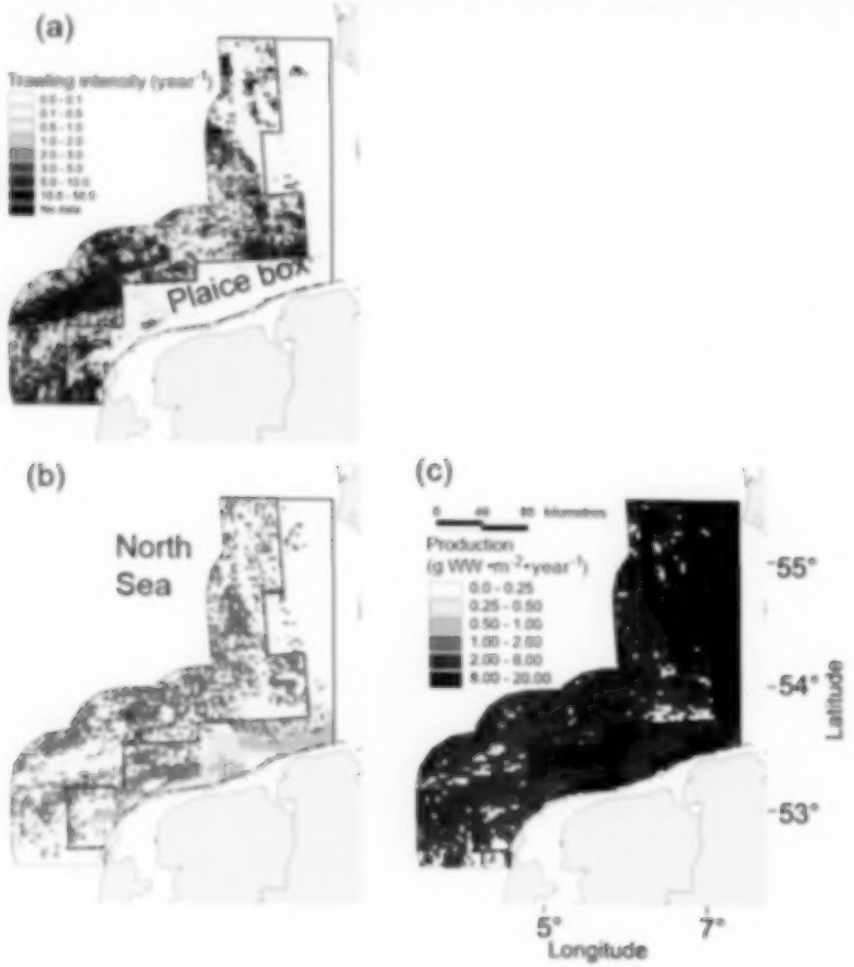

Table 2. Average modelled benthic production in and outside the plaice box for four trawling effort estimates.

	Average trawling frequency (year^{-1})		Total production (g·m^{-2})		Production of small, soft-bodied invertebrates (g·m^{-2})	
	Plaice box	Control	Plaice box	Control	Plaice box	Control
Netherlands 2000–2004	0.33	3.00	11.55	7.15	0.36	0.83
International 2000–2004	0.45	3.96	11.27	6.33	0.36	0.69
Netherlands 1990–1995	0.57	4.48	11.25	6.01	0.35	0.64
International 1990–1995	0.93	6.09	10.97	5.22	0.34	0.51

Note: Average trawling frequency describes the average trawling frequency in space and indicates the total fishing effort levels in and outside the place box in the different scenarios.

NS; Hiddink et al. 2006*b*), and this means the proposed mechanism may explain observed distribution patterns of juvenile plaice, but not that alternative hypotheses have been rejected (see below). Thus, the positive relationship between plaice abundance and benthic biomass may be the consequence of the redistribution of plaice to maximize their food intake and results in a gradual shift out of the protected area into the areas that remain open to bottom trawling. This implies that the plaice box was probably not the most appropriate management measure to protect undersized plaice from discarding, as the undersized fish will redistribute so that they reach the highest densities in areas that are fished. The use of other management measures such as a larger mesh size and a decrease of the overall trawling effort could therefore have been more effective tools to achieve a sustainable exploitation of plaice.

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The relationship between plaice size and prey size only explained a small fraction of the observed variation in prey sizes. However, over 80% of prey for all size classes of plaice were smaller than 0.5 g. This means that the conclusions of this paper are unlikely to be sensitive to the exact relationship between body weight of plaice and prey size. It also means that although adult plaice eat larger prey than juveniles, they still eat relatively small prey and are therefore unlikely to move inshore to benefit from the increased abundance of large invertebrates there.

Alternative hypotheses that may explain the decline in plaice stocks and the shift in the distribution of juvenile plaice, such as an increase in water temperature due to climate change, a reduction of eutrophication, a reduction in intraspecific competition, or an increase in predation risk in shallow waters (Van Keeken et al. 2007), have not been examined in this study and remain to be tested, either separately or in concert. Water temperature in the NS has increased considerably since the establishment of the plaice box (Perry et al. 2005) and in the plaice box area in particular (Van Keeken et al. 2007) and may play a role in explaining the changes in distribution patterns of plaice.

Even though bottom disturbance by trawling may improve feeding conditions for plaice, bottom trawling can have undesirable effects on the ecosystem and other commercial fisheries (e.g., on fish species that feed on large invertebrates, such as Atlantic cod). This paper illustrates that closed areas may not be a suitable management measure to protect juvenile plaice, but does not suggest that bottom trawling has a net positive effect on this species, the ecosystem, or the exploitation in general. The net effect of bottom trawling disturbance on plaice populations depends on the balance between the positive effect on food supply of juvenile plaice versus the negative effect of increased juvenile bycatch mortality. The decision to create MPAs should therefore be based on an assessment of the impact of the MPA on all components of the ecosystem, as the impact on different ecosystem components can be different.

Over time, there seems to have been a shift in the southeastern NS from fish that eat large benthic invertebrates (such as Atlantic cod and rays) to ones that eat small worms (such as plaice, dab (*Limanda limanda*), and common sole (*Solea solea*)). It has been well established that the life-history characteristics of large species make them more vulnerable to the effects of exploitation (e.g., Jennings et al. 1999), and as all the large fish tend to eat large food, it is difficult to disentangle the effects of life history and food. Nevertheless, this mechanism could explain that the effect of bottom trawling on the food sources of these animals (negative for species such as Atlantic cod and rays, positive for the small flatfish) contributed to the observed shift in the composition of the fish fauna from large species to smaller, worm-eating species.

A critical assumption of the benthos model for the current study is that there is competition between different size classes of benthic invertebrates and that without disturbance large animals out-compete the smaller animals. There are good indications that competition among benthic invertebrates exists (for an overview see Wilson 1990). The correspondence between observed and modelled size spectra in the NS indicates that our model realistically reproduces these interactions (Duplisea et al. 2002). To date there is no conclusive empirical evidence that production of small benthic invertebrates increases with trawling disturbance, but some studies suggest that such a release from competition due to trawling disturbance may occur (Jennings et al. 2001; Tanner 2003). However, the reason why trawling studies do not commonly find an increase in small benthos with increasing trawling may be that the range of trawling frequencies that is sampled often does not include many (or any) stations with low trawling frequencies ($\leq$0.3 year^{-1}, even though this is often in the most common trawling frequency category (Hiddink et al. 2007)); therefore, the power to detect a hump-shaped relationship is low (e.g., see fig. 5*c* in Hiddink et al. 2006*b*). The current study therefore indicates that it is important that future studies include low disturbance frequencies and that the response of different size classes of animals to disturbance is analysed separately.

Some of the areas in which plaice were collected for stomach content analysis were heavily trawled, which implies that large, hard-bodied benthos may not have been available as prey. This means that the diet of plaice in our analysis may be biased towards small, soft-bodied prey. However, as Rijnsdorp and Vingerhoed (2001) found no differences between the diet of plaice in and outside the plaice box, the diet as recorded in this study probably reflects the true preference of plaice.

Understanding how habitat selection relates to food abundance is central to predicting the effect of closed areas on the distribution of plaice. Little is known about the habitat selection of plaice >1 year old, but there are some indications that plaice aggregate in locations with good feeding conditions (Hinz et al. 2006). In the EC, adult plaice were most abundant at locations where tubeworms (which were abundant in the stomachs) were abundant (Shucksmith et al. 2006). In another gear restriction area in the EC, large plaice were less abundant and experienced slower growth rates than in an adjacent open access area (Blyth-Skyrme et al. 2006). Future studies could elucidate the role of food abundance in determining plaice distribution by examining plaice abundance (Hinz et al. 2003), abundance of relevant food sources, and plaice condition (e.g., as in Hiddink et al. 2005).

In this study, we implicitly assumed that the availability of benthic food sources affects the distribution of the plaice population, and the negative impact of plaice predation on benthic biomass was not considered. Some fish predators exert a top-down control in benthic food webs (Worm and Myers 2003), and it has been argued that changes in predation pressure by fish in the North Sea due to their exploitation have caused changes in the benthic invertebrate community (Frid et al. 1999; Heath 2005). In such a situation, the current benthic model would not provide accurate predictions of the level of benthic production because fish predation is likely to affect the biomass and size distribution of the benthos. Adding a predator to the benthos model may solve this problem.

Our results support the hypothesis that bottom disturbance may improve the feeding conditions for small-mouthed commercial fish species, such as plaice, that feed on small-sized benthic invertebrates. Bottom trawling may lead to an increase of small, soft-bodied organisms at the expense of

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large fauna, and plaice mostly eat these smaller, soft-bodied species. If juvenile plaice aggregate at the locations with the best feeding conditions, they will follow the fishery, which leads to the conclusion that the plaice box may not have been the most appropriate management measure to protect undersized plaice from discarding.

The general implication of this study is that in fisheries management, MPAs are not necessarily the preferred tool to achieve a specific management objective and that when deciding which tool(s) to use, the ecology of the target species should be taken into account.

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Annual variation in habitat-specific recruitment success: implications from an individual-based model of Lake Michigan alewife (*Alosa pseudoharengus*)

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Abstract: The identification of important spawning and nursery habitats for fish stocks can aid fisheries management, but is complicated by various factors, including annual variation in recruitment success. The alewife (*Alosa pseudoharengus*) is an ecologically important species in Lake Michigan that utilizes a variety of habitats for spawning and early life growth. While productive, warm tributary mouths (connected to Lake Michigan) may contribute disproportionately more recruits (relative to their habitat volume) to the adult alewife population than cooler, less productive nearshore habitats, the extent of interannual variation in the relative contributions of recruits from these two habitat types remains unknown. We used an individual-based bioenergetics simulation model and input data on daily temperatures to estimate alewife recruitment to the adult population by these different habitat types. Simulations suggest that nearshore lake habitats typically produce the vast majority of young alewife recruits. However, tributary habitats may contribute the majority of alewife recruits during years of low recruitment. We suggest that high interannual variation in the relative importance of habitats for recruitment is a common phenomenon, which should be considered when developing habitat management plans for fish populations.

Résumé : L'identification des habitats importants de frayère et de nourricerie pour les stocks de poissons peut améliorer la gestion des pêches, mais elle est compliquée par divers facteurs, dont la variation annuelle du succès du recrutement. Le gaspareau (*Alosa pseudoharengus*) est une espèce d'importance écologique au lac Michigan qui fraye et se développe dans des habitats multiples. Les embouchures productives de tributaires plus chauds (reliés au lac Michigan) peuvent fournir une quantité disproportionnée de recrues (par rapport au volume représenté par ces habitats) à la population adulte de gaspareaux par comparaison aux habitats plus frais et moins productifs près des berges; cependant, l'importance de la variation interannuelle des contributions relatives de ces deux types d'habitat reste inconnue. Dans un modèle de simulation bioénergétique basé sur l'individu, nous utilisons les températures journalières comme données d'entrée pour estimer le recrutement de gaspareaux vers la population adulte à partir de ces différents types d'habitat. Les simulations laissent croire que les habitats le long des berges fournissent généralement la très grande majorité des recrues de gaspareaux. Cependant, les années de faible recrutement, il se peut que les tributaires produisent la majorité des recrues. Nous croyons que la forte variation interannuelle de l'importance relative des habitats pour le recrutement est un phénomène commun dont on doit tenir compte lors de l'établissement de plans de gestion pour les populations de poissons.

[Traduit par la Rédaction]

Introduction

The identification and subsequent protection of habitats that support production of early life stages is critical for the effective management of fish populations. Several initiatives (e.g., the Sustainable Fisheries Act) directly or indirectly address the need for classification of habitats that contribute disproportionate numbers of individuals to adult fish popula-

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tions, either on an areal basis (i.e., nursery habitats; Beck et al. 2001, 2003) or in total (i.e., effective juvenile habitats; Dahlgren et al. 2006). While a focus on spatial differences is appropriate when discriminating among habitats, recruitment dynamics and early life processes of fish also are characterized by high temporal variation (e.g., Hjort 1914; Cowan and Shaw 2002). As such, habitat-specific production rates of juvenile fish are likely to vary over time (e.g., Hilborn et al. 2003; Berkeley et al. 2004; Schindler et al. 2006). Therefore, even the most productive habitats will experience periods of low recruitment (e.g., Peterman et al. 2003).

There is a growing recognition of the biocomplexity of fish populations (Hilborn et al. 2003; Ruzzante et al. 2006). Many fish stocks are characterized by spatially and temporally distinct substocks, which contribute differentially to adult populations and fisheries (Iles and Sinclair 1982; Ruzzante et al. 2000; Andrews et al. 2006). In fact, studies suggest that relative habitat-specific recruitment can vary at annual (e.g., Berkeley et al. 2004) and even decadal (e.g., Hilborn et al. 2003) time scales. Such phenomena imply that management plans that prioritize conservation of the seemingly fittest substocks and most productive habitats are inadequate, and instead, for many systems fish populations and habitats should be managed to maximize genetic (e.g., Ryman et al. 1995) and environmental diversity, respectively.

While it is apparent that the relative contributions of specific habitats to system-wide fish recruitment can vary interannually, mechanistic understanding of such phenomena is lacking. Herein, we present a mechanistic, individual-based model (IBM) to explore interannual variation of both nursery and effective juvenile habitats. An IBM framework is appropriate for depicting annual variation in habitat-specific recruitment because (*i*) it accounts for individual phenotypic variation and allows for movement among habitats; (*ii*) it can bridge the gap between temporally dynamic models of fish populations and static analyses of fish–habitat linkages (Minns et al. 1996); and (*iii*) it can provide mechanistic-derived estimates of habitat-specific recruitment when empirical relationships between habitat-specific recruitment and annual measures of physical and biological factors are unavailable.

Our model is based upon the Lake Michigan alewife (*Alosa pseudoharengus*) population. While alewives are exotic in Lake Michigan, they constitute the forage base for economically important salmon and trout. Further, the Lake Michigan alewife population is a useful model in that the stock biology (Norden 1967; Brown 1972) and habitat utilization (Goodyear et al. 1982; Höök et al. 2007) of this clupeid are similar to many exploited populations throughout the world. Alewife year-class strength in the Great Lakes is positively correlated to summer temperatures (O'Gorman et al. 2004; Madenjian et al. 2005). This temporal association suggests that recruitment success also may vary among habitats, with warmer habitats yielding more young-of-year (YOY) alewives. In fact, field studies during 2001 and 2002 indicate that (*i*) densities of young alewife are greater in drowned river mouth lakes, which are smaller, warmer, and more productive than cooler, nearshore areas of Lake Michigan (Höök 2005) and (*ii*) because of a better growth environment, earlier hatching dates, and size-dependent over-winter mortality, tributary habitats likely yield more young alewife (on a volumetric basis; Höök et al. 2007). Nonetheless, the volume of water contained in nearshore areas of Lake Michigan is at least two orders of magnitude greater than all tributary habitats combined (Höök 2005), and chemical analyses of otolith cores suggest that the majority of alewife recruits in 2002 (age-1 fish in spring) actually inhabited nearshore Lake Michigan during early life (Dufour et al. 2005). However, the 2002 growing season was relatively warm, so habitat-specific contributions estimated for this year may not be indicative of cooler years.

Materials and methods

Model description

Here, we present an overview of the model (details of the model are presented in the Supplemental Materials[1]). The model simulates YOY alewife emergence, growth, survival, and movement throughout Lake Michigan and tributary habitats over a 180-day period (1 May to 27 October). Although the model describes individual-level dynamics of YOY alewives, the model actually tracks 20 000 "super-individuals", with each super-individual potentially representing a multitude of individual fish (e.g., Scheffer et al. 1995). In addition to the number of individual fish represented by a super-individual, other individual-level variables include length (mm), weight (g), percentage of weight composed of storage tissue, percentage of weight composed of structural tissue, and location. The model progresses at daily time steps, and individual level variables are updated each day. The model represents a simplified version of Lake Michigan and surrounding tributary habitats as 10 discrete compartments: two offshore compartments (0–1), four nearshore compartments (2–5), and four tributary compartments (6–9; Fig. 1). Each compartment represents a well-mixed epilimnion (top 10 m of water column) of the corresponding habitat, with homogeneous temperatures, water clarities, and prey densities.

The primary inputs for the IBM are year-specific estimates of daily Lake Michigan water temperatures from a physical model (Croley 1989, 1992; Croley and Assel 1994; Fig. 2). Croley and Assel's (1994) temperature model predicted lake-wide average daily water temperatures at the surface and multiple depths based on observed measures of cloud cover, humidity, wind velocity, and air temperature at various locations. Model predictions closely match observed Lake Michigan surface temperatures (Croley 1992), and the model has been used to model environmental effects on alewife dynamics (Madenjian et al. 2005). Based on a plethora of measured temperatures (Schwab et al. 1999; Höök et al. 2004, 2007), we developed equations to relate daily mean surface temperature

[1] Supplementary data for this article are available on the journal Web site (cjfas.nrc.ca) or may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Building M-55, 1200 Montreal Road, Ottawa, ON K1A 0R6, Canada. DUD 3766. For more information on obtaining material refer to cisti-icist.nrc-cnrc.gc.ca/cms/unpub_e.html.

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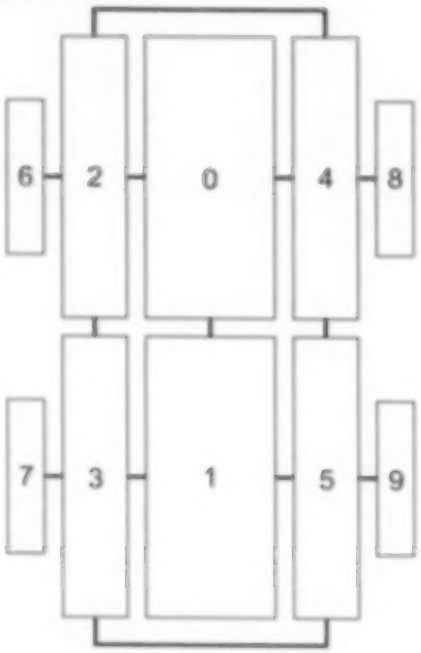
Fig. 1. Diagram of the spatial distribution of habitat compartments represented within the individual-based model. Compartments 0–1 represent offshore Lake Michigan habitats; 2–5 represent nearshore Lake Michigan habitats; and 6–9 represent tributary habitats. Thick connecting lines demonstrate how individual alewives can move between habitat compartments.

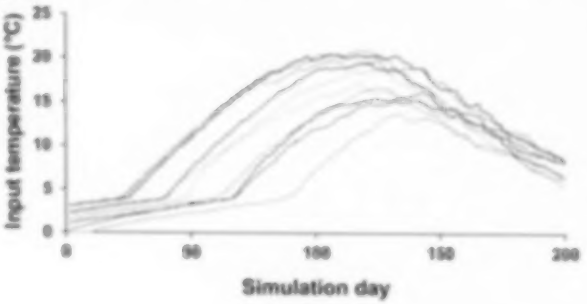


in Lake Michigan to daily, habitat-specific, mean epilimnetic temperature in each of the 10 model compartments (see Supplemental Materials[3]).

In addition to temperature, water clarity and prey densities also vary among habitat compartments. Based on field observations, water clarity in tributary mouths is generally much lower than that in Lake Michigan. Further, as YOY alewives primarily feed on zooplankton, the model depicts three microzooplankton types (copepod nauplii, rotifers, and *Dreissena* spp. veligers) and four macrozooplankton size categories. Prey categories vary in length (and hence vulnerability to gape-limited predators), mass, energy content, and seasonal density (see Supplemental Materials[3]).

In Lake Michigan, adult alewives spawn and larvae subsequently emerge over an extended time period (late May to early August; Jude et al. 1981*a*, 1981*b*; Höök et al. 2007) and in a variety of habitats (Wagner 1972; Goodyear et al. 1982; Mansfield 1984). In each nearshore and tributary habitat compartment (2–5 and 6–9, respectively; see Fig. 1), 2500 super-individuals emerge over a 41-day period. The actual number of super-individuals emerging each day was determined from a parametric distribution function, with a mean at the midpoint of the 41-day emergence period (the day of peak emergence). Each year the same total number of individuals emerged in each habitat compartment, but time of emergence varied among years. Peak emergence dates in each compartment are determined each year as a function of habitat-specific temperatures and time of year.

Fig. 2. Daily temperature inputs from Croley's (1989 and 1992) temperature model for 1992–2002. Each line represents a specific year.



Although the same number of super-individuals emerges in each habitat compartment, super-individuals emerging in different compartments initially represent different numbers of individual alewives (based on field observations of larval alewife densities and the volume of water represented by each habitat compartment). Therefore, super-individuals emerging in nearshore habitats initially represent substantially more individual alewives than super-individuals emerging in tributary habitats.

Model alewives emerge at 4 mm total length (Auer 1982) and 137 µg (6.3% storage tissue and 93.7% structural tissue). Subsequently, individual size and energy composition (Flath and Diana 1985; Stewart and Binkowski 1986; Hewett and Stewart 1989) are updated daily using a bioenergetics framework (Hanson et al. 1997). The model includes a foraging submodel similar to a model presented by Letcher et al. (1996), which tracks alewife encounter rates with their zooplankton prey and uses an optimal foraging approach to model which prey types are included in individuals' diets.

The encounter rate of an individual YOY alewife with a particular zooplankton prey type is a function of the individual fish's total length and swimming speed (a function of size and temperature; Stewart and Binkowski 1986; Klumb et al. 2003), water clarity in the habitat, and density and individual length of the prey type. Time spent attacking and handling prey reduces the time an individual fish can search, attack, and handle other prey; thus, individual alewives only include prey items that should maximize their net daily energy intake (Charnov 1976; Stephens and Krebs 1986). Prey-specific capture success and handling time are dependent on individual fish length, and consumption is limited by gape diameter. After determining which prey to include in an individual's diet (based upon optimal foraging criteria), the number of prey items consumed by an individual fish is randomly selected (from a binomial distribution). However, daily consumption is constrained to not exceed the physiological maximum daily consumption rate (Stewart and Binkowski 1986; Letcher et al. 1996, 1997). Individual alewives lose energy through respiration, egestion, excretion, and specific dynamic action (Stewart and Binkowski 1986; Klumb et al. 2003), and daily energy gain (and tissue growth) is calculated as the difference between daily energy consumed and energy lost.

Mortality occurs through predation and starvation and is

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evaluated daily. Predation mortality declines with increasing size (Mansfield and Jude 1986) and is higher in tributary habitats than in other compartments (based on unpublished trawling and acoustics surveys; T. Höök, D. Mason, and D. Krueger, University of Michigan and NOAA's Great Lakes Environmental Research Laboratory, Ann Arbor, Michigan). Starvation mortality only occurs if the percentage of body mass composed of storage tissue declines below a size-specific threshold.

Each day, individual alewives potentially move (passively or actively) to adjacent habitat compartments (Fig. 1). Potential movements are evaluated in two stages: (*i*) individuals are randomly assigned to either move or remain in their current habitat box as a function of length (i.e., smaller individuals were more likely to move; Höök et al. 2006), and (*ii*) if individuals move, then the direction of movement (i.e., new habitat compartment) is randomly assigned. The model assumes that individuals moving within Lake Michigan are most likely to move in the direction of prevailing cyclonic water currents (i.e., counterclockwise in the Northern Hemisphere; Beletsky et al. 1999).

Model simulations

We simulated YOY alewife dynamics in Lake Michigan habitats for 11 years (1992–2002). The model was initiated each year with daily temperature inputs from the physical model (Fig. 2; Croley 1989, 1992; Croley and Assel 1994), which were used to assign habitat-specific daily temperatures to each of the habitat compartments. Primary model outputs were lengths, weights, and energy densities of individuals alive on simulation day 180 (27 October), as well as individuals' birth dates and natal and current habitats.

We evaluated habitat-specific recruitment as the number of individual alewives ≥60 mm at the end of simulations (27 October). In general, smaller fish tend to have lower energy stores and higher weight-specific respiration rates and are therefore less likely to survive extended periods of limited feeding. Alewives in the Great Lakes likely feed little during winter, as evidenced by large decreases in somatic tissue energy densities from late fall to spring (Flath and Diana 1985). For this reason, several authors have suggested that YOY alewives in the Great Lakes must reach some minimum size to survive winter (Brown 1972; O'Gorman and Schneider 1986; Bergstedt and O'Gorman 1989). Collections of yearling alewives suggest that the minimum length of yearling alewives in spring is 65 mm (Höök et al. 2007). Thus, allowing for the possibility of limited overwinter growth, we assumed that YOY alewives must reach a minimum length of 60 mm by the end of the simulation to survive winter.

Model analysis

To explore the effects of stochastic components of the simulation model on annual size distributions and habitat-specific contributions, we executed the model multiple (at least five) times for selected years. In addition, to consider how model assumptions regarding temporal and spatial patterns of zooplankton densities influenced final size distributions and habitat-specific contributions, we ran model simulations assuming that zooplankton densities were uniform over time and across habitat compartments. Two zooplankton densities were used: (*i*) zooplankton densities set at 1.5 times minimum levels (see Supplemental Materials B, Table SB2[3]) and (*ii*) zooplankton densities set at three times minimum levels. Finally, we executed additional simulations to consider how certain model assumptions (i.e., predation mortality, habitat-specific temperatures, movement, hatching dates, and size threshold to survive winter) influenced final size distributions of simulated alewives and habitat-specific contributions of alewife recruits (see Supplemental Materials[3]).

To analyze our model outputs, we discriminated among three classes of individual alewives: (*i*) Lake Michigan residents, (*ii*) migrants (i.e., alewives born in tributaries that subsequently moved into Lake Michigan), and (*iii*) tributary residents (i.e., alewives born in tributary compartments that remained in natal habitat compartments throughout the simulation). Further, to consider model dynamics, we focused on three representative years: 1995 (an average thermal year), 1996 (the coldest year), and 1998 (the warmest year). To facilitate comparisons across years and habitats, we also evaluated early-life vital rates (daily instantaneous growth (G) and mortality (Z)) for individuals who emerged in different habitat compartments during 1995, 1996, and 1998. For each super-individual, we calculated growth and mortality rates from the day of emergence until 35 days after emergence. We chose this time period because it roughly corresponds to recent field-derived estimates of alewife growth and mortality rates (see Höök 2005).

Model corroboration

We used a pattern-matching approach (Grimm et al. 2005) to corroborate model outputs. First, we compared size distributions of simulated alewives at the end of the 2002 model run with size distributions of YOY alewives captured in nearshore Lake Michigan and Muskegon Lake (a drowned river mouth lake) during October and November 2002; we also compared vital rates of simulated alewives with vital rates estimated for larval alewives captured during summer 2001 and 2002 in nearshore Lake Michigan and Muskegon Lake (see Höök 2005). Second, we compared model predictions of alewife recruitment (number of individuals reaching >60 mm total length during year t) with observed recruitment success (from US Geological Survey, Great Lakes Science Center's fall bottom trawl surveys). We calculated observed recruitment success as recruitment per unit of spawner biomass; recruitment is defined as biomass of age-3 alewives during year $t + 3$, and spawner biomass is estimated as adult biomass during year t (see Madenjian et al. 2005). Finally, we compared simulated habitat-specific contributions of surviving alewives during 2002 with estimates of habitat-specific contributions derived from isotopic signatures in age-1 alewife otolith cores (Dufour et al. 2005).

Results

In general, IBM simulations suggested that during 1992–2002, the majority of alewives recruiting to the adult Lake Michigan population (YOY longer than 60 mm on simulation day 180) emerged in Lake Michigan proper (Fig. 3). On average, 5.1×10^8 alewives per year were large enough

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Table 1. Mean lengths (mm) and dates of birth of three classes of young-of-year (YOY) alewives (*Alosa pseudoharengus*) alive on simulation day 180 (27 October), with mean daily temperature inputs for 1992–2002.

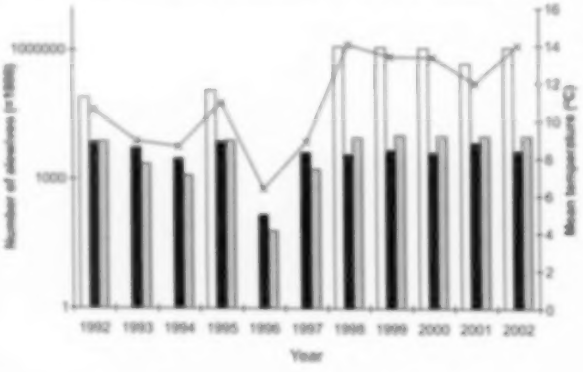
		Mean length (mm)			Mean birth day		
Year	Mean temp. (°C)	Lake Michigan residents	Tributary – Lake Michigan migrants	Tributary residents	Lake Michigan residents	Tributary – Lake Michigan migrants	Tributary residents
1992	10.55	42.8	73.8	85.1	72	55	55
1993	8.89	23.8	57.9	76.0	96	62	62
1994	8.59	20.6	49.9	66.5	100	65	64
1995	10.92	44.1	75.3	84.6	75	57	58
1996	6.37	16.5	32.4	43.9	101	71	67
1997	8.87	22.1	53.6	71.4	99	65	64
1998	14.05	82.9	97.6	87.3	57	47	48
1999	13.40	80.5	94.0	85.9	58	48	48
2000	13.34	80.8	94.4	86.7	57	47	47
2001	11.96	59.2	85.1	86.8	69	54	55
2002	13.99	80.3	95.1	86.2	59	48	48

to survive the winter, out of which 5.0×10^5 emerged in Lake Michigan habitat compartments. However, given that the volume of water contained in nearshore Lake Michigan is more than two orders of magnitude greater than that in all tributary mouths combined (Hook 2005), on a volumetric basis, tributary mouths were roughly twice as productive as nearshore habitats. Further, during relatively cold years, alewife recruitment was much lower, and relative contributions of tributary habitat compartments increased greatly. In fact, although 98% of survivors (from 1992–2002) emerged in Lake Michigan, the annual percent contribution of survivors from Lake Michigan ranged from 0% to 99% (mean = 60%; Fig. 3). Of those fish alive on simulation day 180, migrants and tributary residents tended to have emerged earlier than Lake Michigan fish (Table 1). In addition, migrants and tributary residents tended to be larger than fish that spent their whole life in Lake Michigan (Table 1; Fig. 4).

Survival rates and relative contributions of recruits varied across habitat types (Lake Michigan versus tributaries) and specific habitat compartments (Table 2). On average, individuals emerging in tributaries were more likely to recruit to the adult population. However, during warm years (e.g., 1998), survival rates (individual probability of recruitment) were actually higher for individuals emerging in Lake Michigan (Table 2 and Fig. 5). Not surprisingly, individuals born early tended to grow to larger sizes by the end of simulations (Fig. 5). In turn, during cold years, such early-emerging individuals were most likely to survive the growing season and subsequently recruit to the population (i.e., reach 60 mm by the end of the simulation). However, during warm years of fast growth, late-emerging individuals risked mortality for a relatively short time period and simultaneously were able to obtain a sufficient size by the end of the simulation. As a consequence, during warm years, recruitment success was actually greatest for late-emerging individuals (Fig. 5).

During 1995 and 1996, early-life growth was highest for individuals emerging in tributary habitats, but during warm years, average growth rates were higher in Lake Michigan compartments (Table 3 and Fig. 6). Relationships between time of emergence and early-life growth were also apparent, and during 1995 and 1996, individuals emerging later grew slower (Fig. 6). Differences in early-life mortality rates among habitat compartments were less dramatic. Nonetheless, in 1996, early-life mortality rates were noticeably higher in Lake Michigan habitat compartments (Fig. 6). During warm years, such as 1998, there was no apparent temporal relationship between day of emergence and early-life survival. However, during cooler years, mortality rates tended to be higher for late-emerging individuals (Fig. 6). For individuals emerging at the same time, growth and mortality rates were more variable in tributaries than in Lake Michigan; thus early-life *G*/*Z* values also tended to be more variable in tributary habitats (Fig. 6).

Fig. 3. Numbers of young-of-year (YOY) alewives (*Alosa pseudoharengus*) >60 mm on simulation day 180 (27 October), with mean daily temperature inputs (lines) for 1992–2002 (open bars, Lake Michigan; solid bars, tributary residents; gray bars, tributary – Lake Michigan migrants). Note logarithmic scale for left-hand *y* axis.



Repeated simulations suggested that stochastic components of the model have only minor influences on model outputs. Assumptions regarding zooplankton densities clearly influenced final length distributions and habitat-specific contributions (Table 4). However, qualitative differences among habitats in growth and recruitment success were maintained when zooplankton densities were set at uniformly low or high values (Table 4). Similarly, while relaxation of certain model assumptions (relating to predation mortality, habitat-specific temperatures, movement, hatching dates, and the

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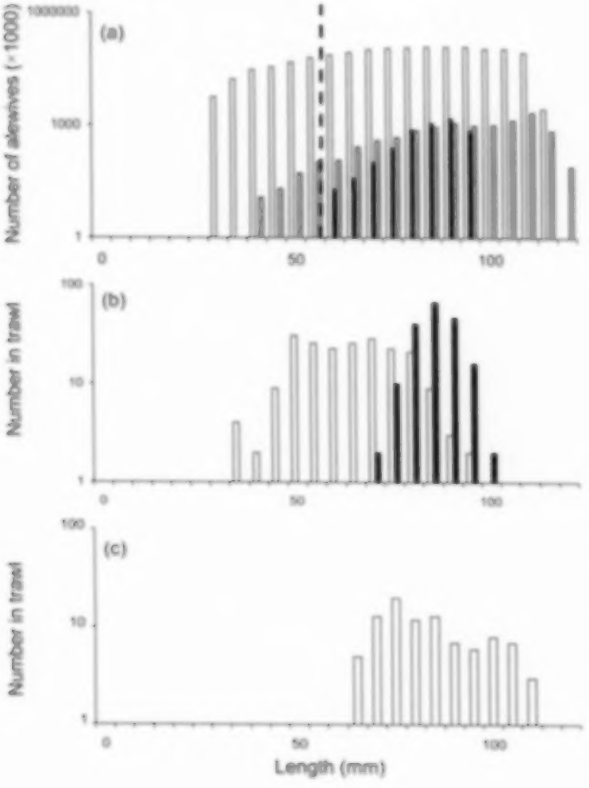
Fig. 4. Alewife (*Alosa pseudoharengus*) length distributions. (*a*) Lengths of three classes of individual alewives (open bars, Lake Michigan; solid bars, tributary residents; gray bars, tributary – Lake Michigan migrants) alive on day 180 during a 2002 model simulation. The broken line represents the 60 mm length threshold that individuals must reach to survive winter. (*b*–*c*) Comparison between length distributions of young-of-year (YOY, age-0; panel *b*) and yearling (age-1; panel *c*) alewives caught by bottom and midwater trawls in nearshore Lake Michigan (open bars) and Muskegon Lake (solid bars) during October–November 2002 and April–June 2003, respectively (data from Höök et al. 2007). Note logarithmic scale for *y* axes.



size threshold to survive winter) influenced quantitative model predictions, qualitative conclusions of our model simulations (i.e., high interannual variation in habitat-specific recruitment) were robust to these individual assumptions (see Supplemental Materials[3]).

Model outputs are consistent with empirical field observations. Lengths of simulated alewives at the end of the 2002 model run were more variable and on average greater than lengths of alewives captured in nearshore Lake Michigan and Muskegon Lake during October and November 2002. However, model- and field-derived length distributions largely overlapped (Fig. 4). In comparing model predictions of alewife year-class strength with recruitment success indexed by US Geological Survey – Great Lakes Science Center's fall bottom trawl surveys, we were only able to compare nine pairs of data (no data for 1995 and 1997).

Table 2. Survival rates of alewives (*Alosa pseudoharengus*) to the end of the simulation and over winter. Mean survival rates are presented for individuals emerging in eight habitat compartments during three representative simulation years.

		Simulation year		
Natal habitat compartment	Simulation timeframe*	1995	1996	1998
Nearshore Lake Michigan habitats				
2	End	0.005	0.000	0.025
	Over winter	0.000	0.000	0.016
3	End	0.013	0.000	0.029
	Over winter	0.001	0.000	0.027
4	End	0.016	0.000	0.032
	Over winter	0.002	0.000	0.029
5	End	0.018	0.000	0.029
	Over winter	0.006	0.000	0.028
Tributary habitats				
6	End	0.014	0.000	0.018
	Over winter	0.008	0.000	0.017
7	End	0.018	0.003	0.015
	Over winter	0.015	0.000	0.015
8	End	0.019	0.002	0.017
	Over winter	0.017	0.000	0.017
9	End	0.018	0.004	0.007
	Over winter	0.017	0.001	0.007

*End, the proportion of individuals alive on simulation day 180, 27 October; Over winter, the proportion of individuals alive and >60 mm on simulation day 180, 27 October.

Nonetheless, for these 9 years, model predictions of recruitment success were significantly and positively correlated with empirical measures of year-class strength ($r = 0.58$). Finally, our model estimates of habitat-specific contributions to the 2002 year class were similar to estimates based on isotopic analysis of otolith cores from age-1 alewives captured during 2003. Chemical analyses of otoliths by Dufour et al. (2005) suggested that out of 41 yearling alewives, only one showed an isotopic signature somewhat consistent with early life in a drowned river mouth lake. Thus, otolith analyses suggested that 0%–2.4% of alewife survivors spent early life in a drowned river mouth lake (Dufour et al. 2005). This range is consistent with our model estimate that 1.3% of alewife survivors during 2002 emerged in drowned river mouth lakes.

Discussion

With a rather straightforward model and a single annual input variable (i.e., temperature), model simulations predicted high interannual variation in estimates of relative habitat-specific recruitment success of alewife in Lake Michigan. Similar phenomena occur in nature and are potentially common (e.g., Hilborn et al. 2003; Berkeley et al. 2004). We suggest that high interannual variation in relative habitat-specific recruitment success may particularly be common for stocks at the latitudinal extremes of a species' geographical range and for fish populations inhabiting large, heterogeneous systems (e.g., alewives in Lake Michigan). Myers (1998) proposed that year-class strength of Northern Hemisphere fish stocks near the northern edge of their geo-

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Fig. 5. Day of emergence (*x* axis) plotted versus (*a*) total length (mm) at the end of simulations (day 180), (*b*) proportional survival to the end of simulations, and (*c*) over-winter survival for super-individual alewives (*Alosa pseudoharengus*) emerging in Lake Michigan (compartments 2–5; black points) and tributary (compartments 6–9; gray points) habitats during three representative simulation years (1995, 1996, and 1998).

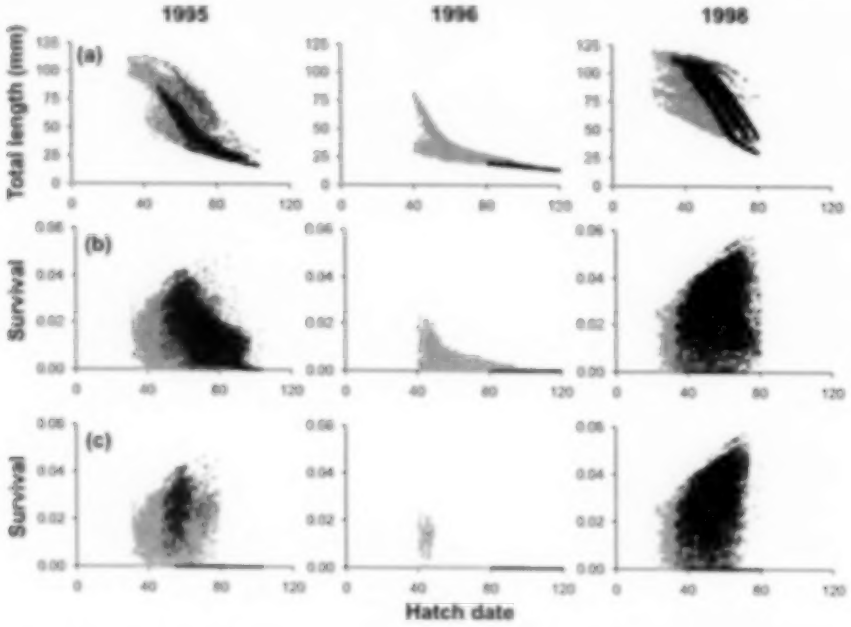

Table 3. Early-life growth rates (mm·day^{-1}; mean ± standard deviation) of simulated super-individuals emerging in eight habitat compartments during three representative simulation years.

	Simulation years		
Habitat compartment	1995	1996	1998
Nearshore Lake Michigan habitats			
2	0.45±0.15	0.19±0.01	0.92±0.07
3	0.74±0.12	0.18±0.01	0.97±0.03
4	0.76±0.10	0.18±0.01	0.97±0.03
5	0.83±0.10	0.18±0.01	0.96±0.03
Tributary habitats			
6	0.90±0.12	0.28±0.09	0.93±0.04
7	0.96±0.05	0.54±0.17	0.84±0.09
8	0.96±0.05	0.48±0.14	0.88±0.07
9	0.95±0.03	0.65±0.17	0.69±0.19

graphic range should be positively related to annual measures of temperature. Shuter and Post (1990) demonstrated that growing season temperatures can influence autumn sizes of YOY fish; thus, summer temperatures largely control the extent of over-winter mortality. Whether or not these processes also translate to annual variation in habitat-specific recruitment success within a system likely depends on the diversity of thermal habitats available. For instance, individuals composing a fish population in a small, isolated lake should experience fairly similar thermal environments during their first year of life; thus, habitat-specific contributions within such a small, homogeneous system will likely vary little. On the other hand, habitat-specific contributions across larger heterogeneous systems, such as a large lake with embayments and tributaries or a river system within a diverse geological landscape, can potentially vary dramatically from year to year (e.g., Hilborn et al. 2003; Berkeley et al. 2004).

While fisheries managers often use quantitative models for short-term (within 1–2 years) predictive purposes, individual-(agent-) based models lend themselves to more exploratory analyses (Bankes 1993, 2002; Jones et al. 2006). Thereby, we view our analysis as a heuristic exercise, and we focus upon qualitative outcomes. Nonetheless, we followed Grimm et al.'s (2005) approach and found that various hierarchical patterns within our individual-based simulations matched empirical observations. Thus, simulation results are likely informative for understanding the ecology of Lake Michigan alewife.

Model simulations indicate that the contributions of specific habitats to overall alewife recruitment vary annually. During cold years of low recruitment, tributary habitats appear to contribute relatively high numbers of young alewives. These predicted annual differences in habitat importance resulted from different temperature inputs (i.e., temperatures are the only annual inputs, and general simulation conclusions were maintained with fixed, uniform zooplankton densities) and size-dependent over-winter mortality. Several authors suggest that YOY alewife must reach some minimum size to survive winter (O'Gorman and Schneider 1986; Bergstedt and O'Gorman 1989; Höök et al. 2007). Ultimately, over-winter survival is likely a function

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Fig. 6. Day of emergence (*x* axis) plotted versus early-life (*a*) instantaneous daily growth (*G*), (*b*) instantaneous daily mortality (*Z*), and (*c*) *G*/*Z* for super-individual alewives (*Alosa pseudoharengus*) emerging in Lake Michigan (compartments 2–5; black points) and tributary (compartments 6–9; gray points) habitats during three representative simulation years (1995, 1996, and 1998).

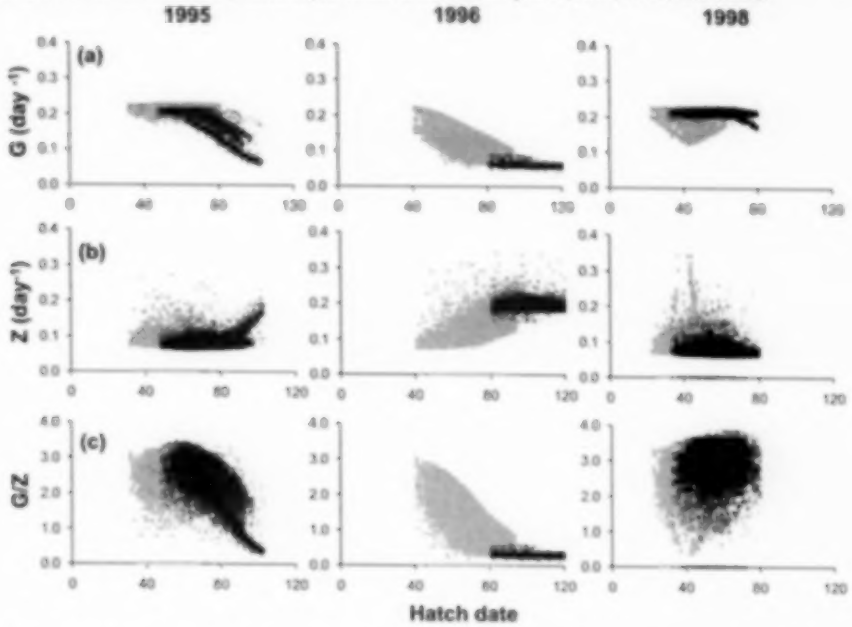

Table 4. Number of survivors (i.e., ≥60 mm) and mean lengths (mm) of three classes of young-of-year (YOY) alewives (*Alosa pseudoharengus*) alive on simulation day 180 (27 October) during a cold (1996), average (1995), and warm (1998) year, with different assumed zooplankton densities.

		No. of survivors			Mean length (mm)		
Year	Prey density	Lake Michigan residents	Tributary – Lake Michigan migrants	Tributary residents	Lake Michigan residents	Tributary – Lake Michigan migrants	Tributary residents
1995	Variable	1.09×10^{5}	8.00×10^{5}	7.48×10^{5}	44.1	75.3	84.6
	Uniform, low	1.24×10^{5}	2.16×10^{5}	1.36×10^{5}	44.6	68.6	62.2
	Uniform, high	1.93×10^{5}	7.32×10^{5}	6.52×10^{5}	45.8	74.0	80.0
1996	Variable	0	6.23×10^{4}	1.49×10^{5}	16.5	32.4	43.9
	Uniform, low	0	2.03×10^{4}	4.51×10^{4}	16.5	31.4	42.7
	Uniform, high	0	4.74×10^{4}	1.18×10^{5}	16.6	32.2	43.3
1998	Variable	1.24×10^{5}	9.38×10^{5}	3.75×10^{5}	82.9	97.6	87.3
	Uniform, low	1.46×10^{5}	2.64×10^{5}	5.23×10^{5}	84.1	94.9	60.5
	Uniform, high	1.95×10^{5}	8.82×10^{5}	3.23×10^{5}	85.7	97.8	81.5

of both an individual's size (which influences mass-specific metabolic rate and thus over-winter energy utilization) and energy density (which determines the amount of stored energy that can be utilized over winter). However, bioenergetics models are generally not well suited to depict energy utilization during a period of resource scarcity; thus, using a bioenergetics approach to model over-winter energy utilization as a function of size and energy density is problematic. Therefore, it was appropriate for us to model over-winter mortality as a function of size, because (*i*) past studies have suggested a relationship between YOY alewife size and over-winter survival, (*ii*) biases likely arise when using a bioenergetics approach to simulate over-winter energy utilization, and (*iii*) simulated alewives maintained a fairly tight relationship between length and energy density (i.e., for predictive purposes, length and energy density were essentially interchangeable). While several studies suggest that the critical size threshold that alewives must reach to survive winter is approximately 60 mm, it is possible that during some years smaller individuals also survive. For instance, Madenjian (unpublished data) found that during May 1994–1995, lake trout (*Salvelinus namaycush*) in Lake Michigan consumed a very small number of alewives between 45 and 60 mm (described in Madenjian et al. 1998). If such small individuals subsequently survive the spring and recruit to the adult population, then a size threshold less than 60 mm would be appropriate. Obviously, a shorter size threshold would alter model predictions of annual habitat-specific re-

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cruitment. However, the general qualitative conclusions of our model simulations (i.e., high interannual variation in habitat-specific recruitment) are robust to the assumption of a much shorter size threshold (e.g., 45 mm).

Some authors suggest that winter severity also may be an important determinant of alewife year-class strength (Brown 1972; Flath and Diana 1985; O'Gorman et al. 2004). However, based on statistical analysis of long-term data sets, Madenjian et al. (2005) demonstrate that although high winter severity had an insignificant negative effect on year-class strength, the influence of this environmental feature on recruitment success of Lake Michigan alewives is far less than growing season temperatures and salmonine predation. Nonetheless, it is possible that annual variation in winter severity could adjust model predictions of relative habitat contributions. Specifically, it is likely that cooler winters would favor over-winter survival of even larger alewives (>60 mm) and thereby inflate interannual variation in relative habitat-specific recruitment success.

Relative recruitment success of alewife varied among years depending on hatch date. Simulated individuals benefited by emerging as late as possible and growing quickly to obtain the ultimate size threshold to survive winter. In other words, late-emerging individuals risk mortality over a shorter time period than early-emerging individuals. During cold years, late-emerging individuals did not recruit, and even though early-emerging alewives may have been susceptible to potential mortality over an extended period, these initial cohorts were able to grow to 60 mm. Such interannual variation in hatch dates of successful recruits is consistent with the notion that interannual variation in population-level recruitment can be minimized by maintaining a population that spawns over an extended time period (e.g., Garvey et al. 2002). Further, as timing of spawning is partially a genetically determined trait, it follows that maintenance of intraspecific genetic diversity should promote sustainability and resilience of fish populations (Ryman et al. 1995; Policansky and Magnusson 1998; Berkeley et al. 2004).

We modeled zooplankton densities deterministically and ran two types of simulations: (*i*) spatially and temporally variable zooplankton densities and (*ii*) uniform zooplankton densities. In simulations with variable zooplankton densities, we relied on density estimates from 2001 and 2002 to depict temporal variation in habitat-specific zooplankton densities. Thus, we did not explicitly model prey depletion and potential density-dependent effects on alewife food availability. Instead, zooplankton density values implicitly incorporated top-down, density-dependent controls, in that density values were derived from natural systems in which fish predation influences temporal patterns of zooplankton densities. This approach for modeling prey densities was appropriate because we only represented young alewife foragers. Other types of fish that compete with young alewives also influence zooplankton community composition and densities. Explicitly accounting for density-dependent prey depletion without tracking the multitude of competitive foragers could lead to inaccurate depictions of zooplankton dynamics. Nonetheless, it is evident from simulations with uniform zooplankton densities that zooplankton densities can influence individual recruitment success. To evaluate the potential impact of model alewife consumption on zooplankton densities, we calculated the total daily consumption of zooplankton prey types in each habitat compartment during 1998 (the warmest year of highest recruitment and presumably the greatest consumption of zooplankton by young alewives). We then compared total consumption with total biomass of available zooplankton. In so doing, we found that model YOY alewives tended to consume a very small proportion of available zooplankton. The greatest percent consumption of zooplankton (~0.13% of available biomass) occurred for large-bodied zooplankton on simulation day 110 in habitat compartment 7. In addition, simulations with spatially and temporally uniform zooplankton densities suggest that temperature (and not zooplankton densities) is the primary determinant of recruitment success.

In conclusion, interannual variation in recruitment success among habitats should be considered when formulating habitat management plans. At the population level, annual recruitment can vary tremendously, confound stock management, and affect long-term population viability. Our analyses suggest that management that increases diversity of habitats may lessen interannual variation in system-wide recruitment success. It would be useful to extend this type of model to follow cohorts for numerous years and thereby better understand the long-term implications of habitat diversity for population sustainability. Further, the use of higher resolution spatial data could facilitate model application at hierarchical habitat scales and thereby improve model predictions. Ultimately, a diversity of habitats may not be critical for maintaining the Lake Michigan alewife population. Our simulations suggest that if growing season temperatures were low for several successive years, then this population would consist almost exclusively of individuals who emerged in drowned river mouth lakes. However, alewives in Lake Michigan are iteroparous, typically mature at age-2, and older individuals (up to age-8) are fairly common in lake-wide collections (Brown 1972; Madenjian et al. 2003). In fact, bottom trawl surveys suggested that the 1998 year class (a very abundant cohort) was still a major contributor to spawning stock in 2004. Thus, more than six consecutive cold years may be necessary for Lake Michigan's alewife population to be dominated by tributary fish, and such a sequence of cold conditions is unlikely in the future. On the other hand, if semelparous or short-lived fish species experience high interannual variation in relative habitat-specific contributions of young fish, then a plethora of habitat types may be necessary to sustain such fish stocks. Thus, for a variety of fish stocks, interannual variation in system-wide recruitment success can likely be minimized by maintaining (or perhaps enhancing) habitat diversity.

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Effect of ambient oxygen on growth and reproduction in Nile tilapia (*Oreochromis niloticus*)

Jeppe Kolding, Lise Haug, and Sigurd Stefansson

Abstract: Growth, size at maturity, gonadosomatic index (GSI), egg size, and absolute fecundity of Nile tilapia (*Oreochromis niloticus*) were significantly affected by oxygen levels (1.5 ± 1.0, 2.8 ± 1.4, and 6.0 ± 1.8 mg·L^{-1}) in a controlled experiment designed to test the hypothesis (D. Pauly. 1984. J. Cons. Int. Explor. Mer, **41**: 280–284) that O_2 is the controlling factor for the transition from juvenile to adult in fish, in general, in the context of phenotypic life history plasticity and "stunting" in tilapias. Size at maturity and the estimated asymptotic size decreased with decreasing O_2 concentration, as predicted by Pauly's hypothesis. All fish matured at the same age (18 weeks old), which is in contrast to conventional definitions of stunting. This finding challenges the suggested plasticity in age at first maturity for tilapia. The results also challenge the hypothesis that stunting is a unique recruitment mechanism, as the smaller fish in the group with low oxygen concentration produced smaller and fewer eggs than the larger fish in the group with high oxygen concentration.

Résumé : La croissance, la taille à la maturité, l'indice gonadosomatique (GSI), la taille des œufs et la fécondité absolue du tilapia du Nil (*Oreochromis niloticus*) sont significativement affectés par les concentrations d'oxygène (1,5 ± 1,0; 2,8 ± 1,4; et 6,0 ± 1,8 mg·L^{-1}) dans une expérience contrôlée visant à vérifier l'hypothèse (D. Pauly. 1984. J. Cons. Int. Explor. Mer, **41**: 280–284) selon laquelle l'O_2 est le facteur qui contrôle le passage du stade juvénile à l'adulte en général, dans le contexte de la plasticité phénotypique du cycle biologique et du « nanisme » chez les tilapias. La taille à la maturité et la taille estimée à l'asymptote sont toutes deux réduites dans les concentrations plus faibles d'O_2, tel que le veut l'hypothèse de Pauly. Tous les poissons atteignent la maturité au même âge (18 semaines) contrairement aux définitions habituelles du nanisme. Cette observation met en doute la plasticité supposée de l'âge à la première maturité chez le tilapia. Nos résultats mettent aussi en doute l'hypothèse selon laquelle le nanisme est un mécanisme particulier du recrutement, car les poissons plus petits dans le groupe soumis aux faibles concentrations d'oxygène produisent des œufs plus petits et moins abondants que les poissons plus gros dans le groupe soumis aux fortes concentrations d'oxygène.

[Traduit par la Rédaction]

Introduction

Age and size at first maturity are of major importance in determining the life history of vertebrates. Basic life-history theory predicts that slow growth will result in a higher age at first maturity (Stearns 1992), which is supported by observations of genotypic and phenotypic correlations between these parameters. However, no set threshold would be expected for single parameters (Stearns and Crandall 1984; Stearns 1992); rather, a certain amount of plasticity in life history, including age at first maturity, has been observed under varying environmental conditions, allowing fitness to be maximized in varying environments. The life-history model suggested by Stearns (1992) describes a trade-off between maturing at a fixed size vs. fixed age, with the shape of the reaction norm varying with species and life history, including seasonality of reproduction. It is, however, largely unknown which environmental factors may act as regulators for the observed phenotypic plasticity.

The Nile tilapia, *Oreochromis niloticus*, belongs to the group of cichlids known as "tilapias" (Philippart and Ruwet 1982; McAndrew 2000), which traditionally support major capture fisheries in most African inland waters (Lowe-McConnell 1982, 2000; Jul-Larsen et al. 2003). Tilapias tolerate a wide range of temperatures, salinities, and water qualities and show high resistance to parasites and diseases (Pullin and Lowe-McConnell 1982; Beveridge and McAndrew 2000). The relative ease and low cost of rearing tilapias have led to their widespread cultivation and introduction in tropical and subtropical areas (Lowe-McConnell 1958, 2000; Blühdorn and Arthington 1990). *Oreochromis niloticus* is the second most important cultured freshwater fish worldwide after carp (Pullin 1991; Food and Agriculture Organisation of the United Nations (FAO) 2006).

Wild tilapias are frequently observed to mature early and breed prolifically in small water bodies, but not in larger lakes (Fryer and Iles 1972; Lowe-McConnell 1982; Kolding 1993), a phenomenon generally referred to as "stunting" (Pullin 1991). This plasticity in growth and size at maturation is believed to be instrumental for their success in different habitats (Lowe-McConnell 2000). Stunting has been

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discussed in the context of phenotypic plasticity and life-history strategies (Iles 1973; Noakes and Balon 1982; Lorentzen 2000), including environmental factors such as light intensity, temperature (Cridland 1962), and food quality and (or) quantity (Gunasekera et al. 1995; Siddiqui et al. 1997). Other factors such as predation (Iles 1973; De Silva and Amarasinghe 1989; Kolding 1993), growth rate (Huet 1972; Iles 1973), condition of the fish (Lowe-McConnell 1958, 1982), and social factors (Jalabert and Zohar 1982; Lowe-McConnell 1982) also have been suggested and partly explored. However, a clear understanding of the causes and the ecological significance of stunting has not been achieved (Pullin and Lowe-McConnell 1982; Brummet 1995; Lorentzen 2000). Iles (1973) suggested that stunting by causing maturation at an earlier age and increasing relative fecundity enables fish to withstand high mortality rates under adverse environmental conditions. As stunting of tilapias is generally observed in small bodies of water, characterized by fluctuating oxygen concentrations, Kolding (1993), based on Pauly's (1984) hypothesis on the relationship between available oxygen and metabolism, suggested that oxygen level might be one proximate factor determining the ontogenetic switch from growth to reproduction in tilapias.

Oxygen is the first limiting physical factor in an aquatic environment (Ross 2000), and fish growth, in general, is oxygen-limited (Pauly 1981, 1984; van Dam and Pauly 1995). Based on the observation that gill area, as a surface, is not growing as fast as the body mass that is to be supplied with oxygen, Pauly (1984) argued that ultimate size in fish is determined by oxygen supply. He suggested a constant, the Q_m–Q_c ratio (where Q_m is the necessary oxygen supply per unit weight at the size of maturation (W_m) and Q_c is the necessary supply for maintenance metabolism at maximum size (W_∞)), as a master regulator determining when an individual will mature. This is summarized here in what may be called a P diagram (Fig. 1), as used by Pauly to develop his argument on the role of oxygen in fish growth (Pauly 1984; van Dam and Pauly 1995). Pauly's hypothesis would explain the relative constancy of the L_m–L_∞ ratio (length at maturity to ultimate length) generally observed in fish (Beverton and Holt 1959; Mitani 1970; Beverton 1992). Further, for any given population, any cause for elevated maintenance metabolism, such as high temperature, osmotic stress, crowding, or low food density, will thus result in stunting, i.e., a reduced ultimate size and a reduced size at maturity (Fig. 1).

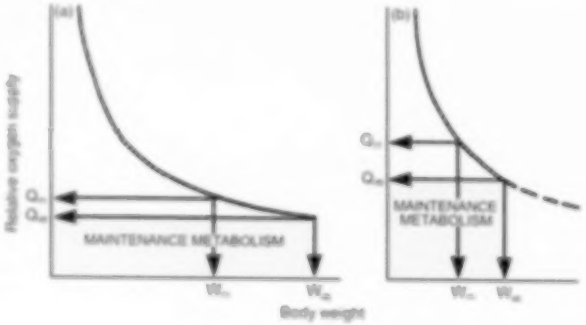
The present study tests the general hypothesis of Pauly (1984), i.e., whether low ambient oxygen concentrations can induce stunting in *O. niloticus*. It also examines Iles' (1973) suggestion that stunting is a response to stress and therefore associated with increased reproductive effort, as expressed in terms of the gonado-somatic index (GSI), egg size, and fecundity.

Material and methods

Fish stock

The fish used in the experiment were juveniles from a broodstock of Nile tilapia (*Oreochromis niloticus*) originating from Lake Manzala in Egypt. The broodstock had been imported from the University of Stirling, Scotland, by the Norwegian Institute of Water Research (NIVA), Oslo, Norway, in 1983 and brought to the University of Bergen in 1993 where the experiment took place.

Fig. 1. P diagram, expressing the interrelationships between internal metabolic oxygen supply at maintenance level (Q_c), oxygen supply near first maturity (Q_m), and the weight of fish. Note that the Q_m–Q_c ratio is constant for two different habitats: (*a*) habitat linked with large asymptotic size and large size at maturity, and (*b*) habitat associated with a small ultimate size and small size at maturity because of higher maintenance metabolism. Note also that the constancy of the Q_m–Q_c ratio can only be imperfectly mirrored in the L_m–L_∞ ratio, which can be derived from W_m and W_∞ (modified from Pauly 1984).



The juveniles used in this experiment were produced in two batches, each originating from a single female, fertilized by different males, to minimize genetic variability. The two batches were released on 2 and 4 August 1995. From 6 August, all juveniles were mixed and reared under the same conditions. In the first week, the fish were reared in one 150 L aquarium, after which they were transferred to a grey, square, fibreglass tank with a rearing volume of 500 L, equipped with a 1000 L·h^{-1} bio-filter pump (Eheim 2217; Eheim GmbH and Co. KG, Deizisau, Germany). The juveniles were fed aquarium feed (Tetra-Min; Tetra GmbH, Melle, Germany) from the day they were released from the mouth of the females until 25 August 1995. From this date until the experiment started on 25 September 1995, they were fed a dry diet (size 1.2 mm pellets; Felleskjøpet Fiskefôr AS, Stavanger, Norway) and aquarium feed (cichlid sticks, Tetra GmbH). At the start of the oxygen treatments, the juveniles were 8 weeks old and the initial mean total length (standard deviation, SD) was 4.75 (0.45) cm.

Experimental design

On 25 September, 781 juveniles were weighed and distributed into six square, grey, fibreglass tanks with open surface and a rearing volume of 200 L (130–131 fish in each tank) in three groups with different oxygen concentrations, with each group consisting of two replicate tanks. The mean oxygen concentrations (SD) in the low, medium, and high oxygen treatments were 18.4% (11.6%), 35.4% (17.7%), and 75.2% (22.2%), corresponding to 1.5 (1.0) mg·L^{-1}, 2.8 (1.4) mg·L^{-1}, and 6.0 (1.8) mg·L^{-1}, respectively. The low and medium O_2 concentrations were chosen as tila-

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pia are known to live and reproduce and are reared in waters with such low oxygen levels (Welcomme 1969), whereas the higher concentration (6 mg·L^{-1}) is above the dose-dependent relationship between O_2 and growth (Brett 1979; Ross 2000). All tanks were supplied with dechlorinated, pH-adjusted (pH = 7.0) fresh water with a mean temperature of 27.6 °C (1.3 °C) at a flow rate of 1.5–6.0 L·min^{-1}, which was adjusted according to the increase in biomass.

Each of the three oxygen groups consisted of two replicate tanks, a 200 L header tank and one bio-filter pump (Eheim 2217; Eheim GmbH and Co.). Each pump drained the two replicate tanks, removing food and faeces from the water, and pumped the water back into the header tank. To increase water flow, an additional pump (same type) was added on 25 October in the high and medium oxygen groups and on 29 October in the low oxygen group. The oxygen concentration was monitored and regulated by a complete oxygen measurement system (E-module; DAN Technique International A/S, Roskilde, Denmark), which regulated electromagnetic valves (Burkert-Contromatic A/S, N-2026 Skjetten, Norway) controlling the supply of atmospheric air, O_2, or N_2 from pressure tubs (Hydrogas, Bergen, Norway). Oxygen probes (Oxyguard International A/S, Blokken 59, DK-3460 Birkerød, Denmark) were placed in the header tanks where monitoring and adjustment of the oxygen concentration occurred. Depending on the desired oxygen level and the biomass in each group, air, N_2, or O_2 diffusers, or a combination of these, were activated. The oxygen concentration in the tanks was further checked manually using Oxyguard Handy MK 111 (Oxyguard International A/S Denmark).

Rearing conditions

Water temperature in the experimental tanks was kept at 27.6 °C (1.3 °C) by immersed heaters in each header tank and by keeping all tanks in a temperature-controlled room. This temperature is within the range of optimal temperatures for *O. niloticus* (Wohlfarth and Hulata 1983; Breine et al. 1996). Temperature was lowered approximately 3–4 °C during sampling days, when half of the water in each tank was replaced. The prescribed temperature was re-established during the following night. Light, with intensity ranging from 150 to 270 lx, was provided by fluorescent tubes positioned in the middle of the room, operated by an automatic timer simulating twilight periods. At the start of the experiment, the light regime was kept constant at 9.5 h light – 14.5 h dark. On 2 November, it was changed to 11.5 h light – 12.5 h dark, which was maintained throughout the experiment. Maximum stocking density in the high oxygen group was 20.7 kg·m^{-3}, which is well within the range recommended for intensive culture of *O. niloticus* (Siddiqui et al. 1991). The fish were fed a commercial dry diet (53% protein, 12% fat, 16% carbohydrate, 8% ash; Felleskjøpet Fiskefôr, A/S). During week 1 (26 September – 2 October), the fish were fed manually three to eight times daily during daylight hours. Beginning in week 2, food was dispensed every 30 min from automatic feeders during daylight hours. Appetite was checked at the end of each day to ensure that the fish were fed to satiation. The groups were fed rations varying from 1.5% to 9.2% body weight·day^{-1}, depending on biomass and growth rate and to minimize negative effects of excess waste feed. Surplus food was siphoned out daily after the last feeding. The fish were not fed on the fortnightly sampling days and during the last two days at the end of the experiment.

Sampling and analyses

Length, weight, and maturity

Total length (to the nearest 0.1 cm) and wet weight (to the nearest 0.01 g) of all fish were measured every 2 weeks. At the same time, 5–15 fish from each tank were sacrificed for determination of sex, gonadosomatic index (GSI), and state of maturity. Investigations of fecundity and egg size were made from these samples on weeks 16 and 18. Immediately after dissection, the gonads were fixed in Karnovsky fixative (Nylund 1985). Sex was determined based on the morphology, size, and color of the gonads (Babiker and Ibrahim 1979). Gonads that could not be be sexed by macroscopic examination were analysed histologically; they were dehydrated, cleared, infiltrated, embedded in Historesin (Leica historesin embedding kit; Leica 702218500), and sectioned (5 µm) using a Leica microtome (Leica Microsystems GmbH, Wetzlar, Germany). Cross sections were dried, stained with Diff quick (Merz and Dade AG, CH-Dudinger, Switzerland), and examined using a light microscope (12× magnification). Sex was determined based on the histological classification of Latif and Saady (1973*a*, 1973*b*). Because low GSI does not necessarily indicate an immature fish, age at 50% maturity was determined from morphological studies of the gonad, rather than from GSI values. Nile tilapia have a very fast reproduction cycle and are able to produce new broods within a few weeks of each other (Peters 1963). However, the readiness to spawn can only be maintained for approximately 1 week, and the ova are subsequently resorbed if appropriate spawning conditions are not available during this period (Peters 1963). The females were classified as mature when oocytes started to be visible, and males when the testis started to become thickened and enlarged (stage 2; Babiker and Ibrahim 1979).

Fecundity and egg size

Fecundity of tilapias can only be reliably assessed when the eggs of an emergent spawn in ripe fish are separated from those of the latent successive spawns (Peters 1963). Size–frequency distributions of oocytes were therefore made of all ripening or ripe ovaries, defined as tense with numerous pear-shaped oocytes (Latif and Saady 1973*b*; Babiker and Ibrahim 1979), to separate ripe and ripening females. Subsamples taken from the middle part of each ovary were weighed to the nearest 0.0001 g, and the size (largest diameter) of the oocytes was measured to the nearest 0.04 mm using a binocular microscope (12× magnification). Thereafter, the egg size and fecundity were determined from ripe females by studying the oocytes in addition to the tenseness of the ovary. Only the most developed modal group (G1 oocytes; Kjesbu 1994), separated by a gap from the following oocyte group, was tested for differences in egg size. To avoid possible differences between different sections of the gonad, subsamples were always taken from the middle part of the lobe.

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Calculations

Specific growth rate (SGR) was calculated according to the formula of Houde and Schekter (1981):

$$(1)\quad \mathrm{SGR} = \left(\exp\left[\frac{\ln(W_2) - \ln(W_1)}{t_2 - t_1}\right] - 1\right) \times 100$$

where W_2 and W_1 are body weights at time t_2 and t_1, respectively, measured in days.

Geometric mean weight (GM) was calculated as

$$(2)\quad \mathrm{GM} = \sqrt{(W_1 \cdot W_2)}$$

where W_1 and W_2 are body weights at time t_1 and t_2, respectively.

Condition factor (CF) was calculated as

$$(3)\quad \mathrm{CF} = \left(\frac{W}{L^3}\right) \times 100$$

where W is body weight (g) and L is total length (cm).

Gonadosomatic index (GSI) was calculated as

$$(4)\quad \mathrm{GSI} = \frac{W_g}{W_{wt}} \times 100$$

where W_g is the weight of the gonad and W_{wt} is the total weight of the fish including gonads.

The absolute fecundity (Bagenal 1978) was calculated as

$$(5)\quad \text{absolute fecundity} = \frac{W_g}{W_s} \cdot N$$

where W_g is the weight of the gonad from which the subsample W_s was taken, and N is number of ripe eggs (most developed modal group) in W_s.

The relative fecundity (Bagenal 1978) was calculated as

$$(6)\quad \text{relative fecundity} = \frac{\text{absolute fecundity}}{W_{wt}}$$

where W_{wt} is the total weight of the fish including the gonads.

The length and age when 50% of the fish were mature, L_m and Age_m, respectively, were calculated as

$$(7)\quad L_m = \frac{b_L}{a_L}$$

and

$$\mathrm{Age}_m = \frac{b_A}{a_A}$$

where a and b are fitted constants of the following logistic model (Gunderson et al. 1980):

$$(8)\quad P_m = \frac{1}{1 + \exp(b - a \cdot x)} \times 100$$

where P_m is percent mature fish at length or age (x).

The effect of oxygen on L_m and Age_m was analysed by fitting the fraction of mature females (per 10 mm intervals or by sampling day) to the logistic model by a nonlinear regression, weighted by the total number of individuals for each size class or sampling day.

The growth parameters L_∞ and K were determined by fitting the length-at-age data to the von Bertalanffy growth function (VBGF; von Bertalanffy 1957):

$$(9)\quad L_t = L_\infty(1 - e^{-K(t - t_0)})$$

where K is the "curvature parameter" determining how fast the fish approaches L_∞ (the asymptotic or ultimate length). Fitting was done using the nonlinear iterative numerical search of the minimum sum of squares (SSE = Σ(observed – predicted)^2) implemented in FiSAT (Gayanilo et al. 1995).

The growth performance index ϕ' was calculated according to the formula of Munro and Pauly (1983) as

$$(10)\quad \phi' = \log_{10}K + 2\log_{10}L_\infty$$

Contrary to L_∞ (dimension: length), and K (dimension: time^{-1}), ϕ' has the dimension of a growth rate (length·time^{-1}) and, hence, can be used to compare different growth performances.

Statistical analyses

To assess for normality of distribution of the measured variables, a Kolmogorov–Smirnov test was performed (Zar 1984). Weight data were $\log_{10}$-transformed prior to analysis. The homogeneity of variances was tested using the Levene's F test (Brown and Forsythe 1974). A nested analysis of variance (ANOVA), with replicates nested within oxygen groups, was applied to calculate the effect of oxygen on weight, length, and condition factor. Regressions of SGR against geometric mean weights were analysed using a covariance analysis (Zar 1984). One-way ANOVA was used to test the following: (*i*) effect of oxygen on GSI in females and males, (*ii*) effect of oxygen on the growth of each sex, (*iii*) differences in growth between females and males in each oxygen group, and (*iv*) differences in length, weight, GSI, egg size, and absolute and relative fecundity of ripe fish in each oxygen group. Significant ANOVAs were further analysed by a Student–Newman–Keuls multiple comparison test to determine differences among experimental groups.

A Kruskal–Wallis one-way ANOVA by ranks test (Zar 1984) was used when testing for differences in length of immature and mature females in each oxygen group and differences between replicates in oxygen concentration for each sampling period. Where significant differences were found, a multiple comparison Kolmogorov–Smirnov two-sample test was applied.

Statistical analyses were performed in Statistica version 5.0 (StatSoft Inc., Tulsa, Oklahoma), and data were considered significant if $p < 0.05$.

Results

Mortality and behaviour

No mortality was observed during the experiment in any group, although oxygen availability influenced behaviour in several ways. Fish in the low oxygen group were often observed positioned close to the submerged water inlet performing "air gulping" at the surface, but this behaviour was

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Table 1. Mean length (cm) of female and male *Oreochromis niloticus* reared under three ambient oxygen concentrations (high, medium, and low).

	Females			Males		
Week	High	Medium	Low	High	Medium	Low
2	6.3±1.23a (3)	6.0±0.53a (3)	5.7±0.62a (6)	6.7 (1)	8.1 (1)	6.3±0.35a (2)
4	7.5±0.29a* (5)	7.5±0.35a (6)	6.5±0.37b (7)	8.1±0.12a (5)	7.6±0.86a (4)	7.0±0.49a (3)
6	9.0±0.27a (5)	8.2±0.54b* (5)	7.7±0.28b* (7)	9.6±0.9a (5)	9.0±0.76a (5)	8.4±0.47a (3)
8	9.9±0.82a (12)	9.1±0.95b* (9)	8.3±0.48c* (13)	10.7±0.99a (7)	10.0±0.57a (11)	8.9±0.68b (7)
10	11.3±1.12a (15)	10.1±1.03b* (11)	9.6±0.74b* (12)	11.8±1.21a (5)	11.4±1.03a (9)	10.5±1.23a (9)
12	12.5±1.15a (14)	11.5±1.35b* (17)	10.1±1.56c* (19)	13.0±1.95a (15)	12.7±1.60a (13)	12.0±1.76a (11)
14	12.9±1.47a* (19)	12.3±1.35a* (18)	11.0±1.21b* (14)	15.1±1.84a (11)	14.7±1.47a (12)	12.6±1.02b (15)
16	13.6±1.59a* (16)	12.6±1.63a* (18)	11.1±1.28b* (18)	15.7±2.45a (14)	15.1±1.59ab (12)	13.7±0.79b (12)
18	14.4±1.49a* (19)	13.3±1.44b* (21)	12.0±1.43c* (20)	17.3±2.81a (9)	15.8±3.50a (9)	14.5±1.86a (10)

Note: Values are means ± standard deviation (SD), number of observations (n) in parentheses. Values not followed by the same letter on the same date are significantly different ($p < 0.05$). Values of females followed by a asterisk (*) are significantly different ($p < 0.05$) from corresponding groups of males.

not observed in the other groups. The fish in the low oxygen group also seemed to have a higher ventilation frequency (although ventilation frequencies were not recorded) and were observed to have a lower food ingestion rate (more uneaten food was found in the tanks despite rations being adjusted to the SGR) compared with the fish in the higher oxygen groups.

Growth

No consistent differences in growth rate between replicate tanks were found (nested ANOVA); hence, replicates were combined in all cases. Length and weight were reduced in response to the lowered oxygen concentrations (Figs. 2*a*–2*b*), with significant differences in length from week 4 and in weight from week 2. Males grew faster than females in all oxygen groups ($p < 0.05$; Table 1). Significant differences were found from week 6 in both the low and medium oxygen groups and from week 14 in the high oxygen group. Consistent differences in growth among females were observed as a consequence of oxygen concentration (Table 1). Among males, differences in growth were only found on weeks 14 and 16 between the high and low oxygen groups (Table 1). However, males in the low and medium oxygen groups were relatively smaller than males in the high oxygen group throughout the experiment.

Specific growth rate (SGR) decreased in all groups throughout the experiment (Table 2), but less in the low oxygen group than in the high oxygen group. By the end of the experiment, the SGR of the low oxygen group was as high as, or higher than, that of the other two groups. Differences were not evaluated statistically because of the low degrees of freedom ($n = 2$, replicate tanks). The decreases in SGR were size-dependent and therefore were not related only to oxygen concentration. The size-specific growth rates (SSGR), obtained by plotting the SGR as a function of geometric mean weight (Fig. 3), showed a similar decline in growth rate with increasing weight for all oxygen groups. No significant differences were found between the slopes of the SSGR (test of parallelism, $n = 18$, $p = 0.591$; Table 3), suggesting that the fish would approach their ultimate size (L_∞) at about the same time. An overall significant difference was found between the intercepts of SSGR, but no significant differences were found in the multiple comparison tests. The high oxygen group was found to have the highest value of ϕ' (Table 4), in accordance with this group reaching the largest size.

Fig. 2. (*a*) Mean length and (*b*) mean weight of *Oreochromis niloticus* under three oxygen concentrations: high, triangles; medium, squares; low, circles. The vertical lines indicating SE may be obscured by symbol; n.s., not significant at $p \geq 0.05$.

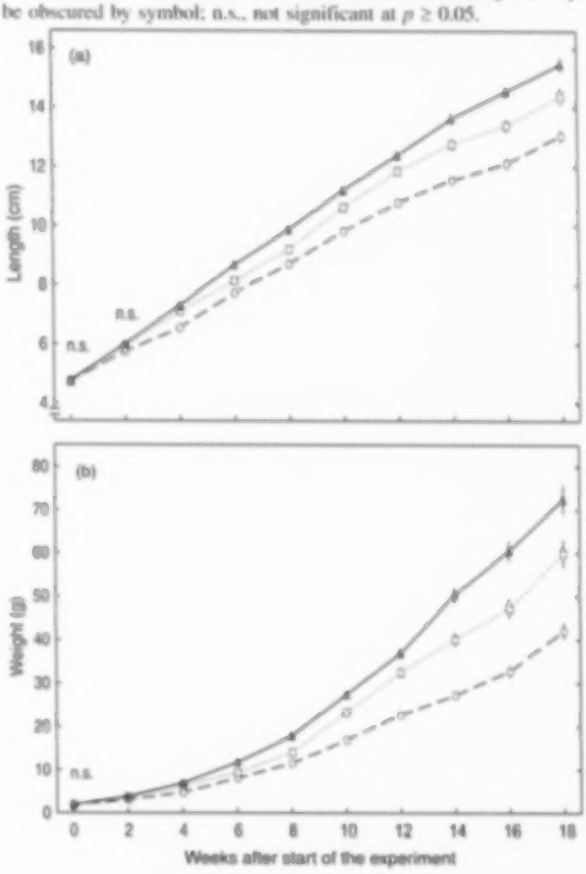

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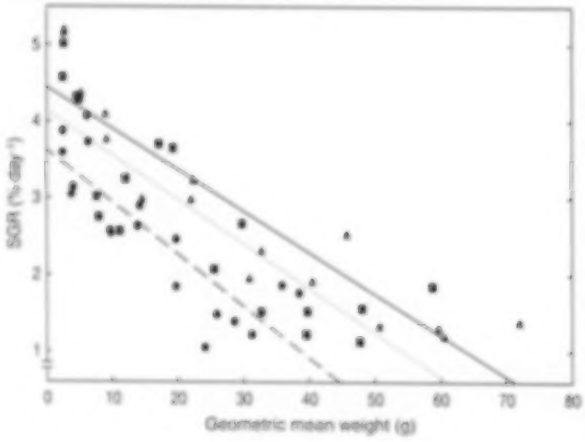
Table 2. Specific growth rate (SGR) of *Oreochromis niloticus* reared under three ambient oxygen concentrations (high, medium, and low).

Week	High	Medium	Low
2	5.18±0.03	4.80±0.31	3.74±0.20
4	4.36±0.02	4.30±0.02	3.09±0.07
6	3.88±0.24	2.83±0.20	3.83±0.21
8	3.04±0.01	2.95±0.49	2.63±0.06
10	3.11±0.18	3.70±0.08	2.76±0.18
12	2.14±0.26	2.37±0.41	2.15±0.44
14	2.22±0.43	1.52±0.001	1.27±0.31
16	1.32±0.02	1.18±0.06	1.31±0.12
18	1.30±0.13	1.71±0.21	1.81±0.07
Average	2.95±1.31	2.81±1.24	2.51±0.93

Note: Values are means ± standard deviation (SD), based on SGR for each replicate ($n = 2$).

Fig. 3. Regression of specific growth rate versus geometric mean of *Oreochromis niloticus* under three oxygen concentrations (high, triangles; medium, squares; low, circles).



Condition factor

The development in condition factor, with an overall increasing trend, was similar in all groups (Table 5). Significant differences in the condition factor occurred only temporarily between the oxygen groups, but where significant differences were observed, condition factors of the low and medium oxygen groups were always lower than the condition factor in the high oxygen group.

Sexual maturity

A significant difference in GSI of the females was only found at week 8, when GSI of the high oxygen group was lower than the GSI of the medium and low oxygen groups (Fig. 4*a*). No significant difference in GSI was found in males (Fig. 4*b*). An increase in GSI appeared in week 8 (i.e., 16 weeks old) for the females and in week 6 (i.e., 14 weeks old) for the males, which by examination of the gonads, coincided with the commencement of sexual maturation. All sampled females and males were mature from week 16 (i.e., 24 weeks old). During the period of the experiment when both mature and immature specimens were observed in the groups (between weeks 8 and 14), the mature females were always larger than the immature females (although the degrees of freedom were generally low for testing, mean lengths were significantly different between mature and immature females for the high and low oxygen groups in week 14).

Table 3. Results from the regression of specific growth rate (SGR) versus geometric mean weight of *Oreochromis niloticus* under three ambient oxygen concentrations (high, medium, and low). The regression equation is given as $y = \alpha + \beta x$.

O_2 group	n	β	$p(\beta)$	α	r^2
High	18	–0.054	<0.001	4.446	0.84
Medium	18	–0.058	<0.001	4.138	0.71
Low	18	–0.068	<0.001	3.623	0.73

No significant difference in age at 50% maturity (Age_m) of females was found (Fig. 5). Age_m in the high, medium, and low oxygen groups were 10.0, 9.3, and 9.7 weeks, respectively, after the start of the experiment (i.e., approximately 18 weeks old). Length at 50% maturity (L_m) of females was significantly higher in the high oxygen group than in the medium and low oxygen groups (10.7, 9.4, and 8.9 cm, respectively; Fig. 6). Thus, the L_m–L_∞ ratios in the high, medium, and low oxygen groups were 0.17, 0.20, and 0.25, respectively, when using the estimated L_∞ values from the VBGF (Table 4).

Reproductive traits

Morphological investigation of gonads and investigation of size-frequency distribution of oocytes from ripening or ripe ovaries made it possible to distinguish 12 ripe fish from both the low and high oxygen groups and seven ripe fish from the medium oxygen group. Among these fish, an overall positive relationship was found between GSI, egg size, and absolute fecundity and increasing oxygen concentration. However, significant differences in GSI, egg size, and absolute fecundity were found only between the low and high oxygen groups (Table 6). No significant difference was found in the relative fecundity between any of the oxygen groups. Significant differences in mean length and weight were found between ripe fish in all groups, i.e., in line with differences observed in the total population.

Discussion

Mortality and behaviour

Generally, *O. niloticus* is able to tolerate dissolved oxygen at concentrations as low as 1 mg·L^{-1}; below this level, they may be able to utilize atmospheric oxygen (Chervinsky 1982; Ross 2000). The absence of natural mortality during the experiment confirmed this high tolerance, although fish in the low oxygen group were often observed performing aquatic surface respiration (ASR), or "air gulping", which is a common behaviour among tropical freshwater fish (Burggren 1982; Kramer and McClure 1982; Ross 2000). ASR probably facilitated the survival of the fish under extreme hypoxic periods in the present experiment. Compensating for low ambient oxygen by ASR and higher ventilation frequency was not considered critical for the testing

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Table 4. Estimated von Bertalanffy growth parameters (L_∞, K) and growth performance index (ϕ') with standard error (SE) of estimates in parentheses, estimated size at 50% maturity (L_m), and the ratio of size at 50% maturity and ultimate length (L_m:L_∞).

O_2 group	L_∞ (cm)	K (year^{-1})	ϕ'	L_m (cm)	L_m: L_∞
High	61.2 (69.9)	0.594 (0.82)	3.35 (1.16)	10.7	0.17
Medium	47.5 (32.7)	0.734 (0.64)	3.22 (0.71)	9.4	0.20
Low	35.2 (11.2)	0.934 (0.40)	3.06 (0.41)	8.9	0.25

Table 5. Condition factor of *Oreochromis niloticus* reared under three oxygen concentrations (high, medium, and low) over a period of 18 weeks.

Week	High	Medium	Low
0	1.68±0.18a (260)	1.69±0.20a (260)	1.67±0.17a (261)
2	1.71±0.24a (261)	1.67±0.26a (261)	1.59±0.18a (257)
4	1.72±0.15a (251)	1.69±0.18b (250)	1.65±0.18c (246)
6	1.73±0.12a (241)	1.65±0.17a (238)	1.70±0.12a (236)
8	1.76±0.11a (230)	1.68±0.11b (231)	1.67±0.09b (227)
10	1.83±0.10a (209)	1.80±0.10b (207)	1.69±0.07c (206)
12	1.81±0.12a (186)	1.79±0.11a (182)	1.70±0.09a (181)
14	1.87±0.10a (154)	1.75±0.08b (152)	1.66±0.08c (151)
16	1.84±0.12a (125)	1.77±0.10a (122)	1.73±0.28a (120)
18	1.83±0.08a (95)	1.81±0.08a (91)	1.78±0.12b (90)

Note: Values are means ± standard deviation (SD) with number of observations, *n*, in parentheses. Values followed by the same letter on the same date are not significantly different ($p < 0.05$).

of Pauly's hypothesis as such stress would also increase maintenance metabolism, as further elaborated below.

Growth: effects of oxygen and sex

Growth and condition factor of both the low and medium oxygen groups were reduced by the lowered oxygen concentrations. This negative relationship between growth rate and oxygen concentration is in accordance with previous findings on tilapia (Tsadik and Kutty 1987; Siddiqui et al. 1991; Teichert-Coddington and Green 1993). However, the absolute differences in growth rates between the medium and high oxygen groups were less than between the low and medium oxygen groups throughout the whole experiment. This suggests that the medium oxygen group (mean concentration of 2.8 mg·L^{-1}) was close to the threshold above which oxygen concentration may have little or no influence on growth. This so-called "critical oxygen level" for *O. niloticus* has been reported to range between 2.5 and 3.1 mg·L^{-1}, depending on factors such as temperature, dietary protein content, ration levels, handling stress, and body weight (Abdel-Magid and Babiker 1975; Fernandes and Rantin 1989; Ross 2000).

Reported ϕ' values for wild *O. niloticus* populations range from 2.36 to 3.11 (N = 16; mean = 2.65; standard error (SE) = 0.06); for farmed *O. niloticus*, ϕ' values range from 2.64 to 3.62 (N = 65; mean = 3.21; SE = 0.03) (Pauly et al. 1988). Thus, with the estimated ϕ' of 3.35, 3.22, and 3.06 in the high, medium, and low oxygen groups, respectively, the growth performance of the fish in the present study was in the upper range of values reported for wild fish, but comparable with growth in farmed fish. It may thus be concluded that oxygen concentration in the high oxygen (control) group allowed the fish to express their full growth potential.

The steady decrease in SGR in all oxygen groups was expected, as the growth rate of fish generally declines with increasing fish size (Brett 1979; Pauly 1981). However, the difference in SGR among different groups also decreased during the experiment, and by the end, the SGR in the low oxygen group was above that of the high oxygen group. This is most likely a consequence of the increased differences in body size, and by adjusting the SGR for size (SSGR), all groups showed similar changes in SGR with size (i.e., parallel slopes), suggesting that they would complete their growth (and approach their ultimate size, L_∞) at around the same age.

Significantly faster growth of males compared with females was found in all oxygen groups. Sexual dimorphism in growth rate in tilapia species is usually expressed in faster growing males, both in aquaculture and in the wild (Lowe-McConnell 1958; Fryer and Iles 1972; Pauly et al. 1988). In some large lakes and dams, however, the sexes have been found to grow at the same rate (Lowe-McConnell 1982). It has been suggested that the slower growth observed in the females is because of the higher cost of reproduction (Wootton 1985) or that mouth-brooding retards growth because of reduced feeding (Huet 1972). However, in our experiment, differential growth started before breeding and was evident at the time of increase in GSI (weeks 6–8). This suggests that physiological changes associated with sexual maturation are at least partly responsible for the growth differences observed between male and female tilapia.

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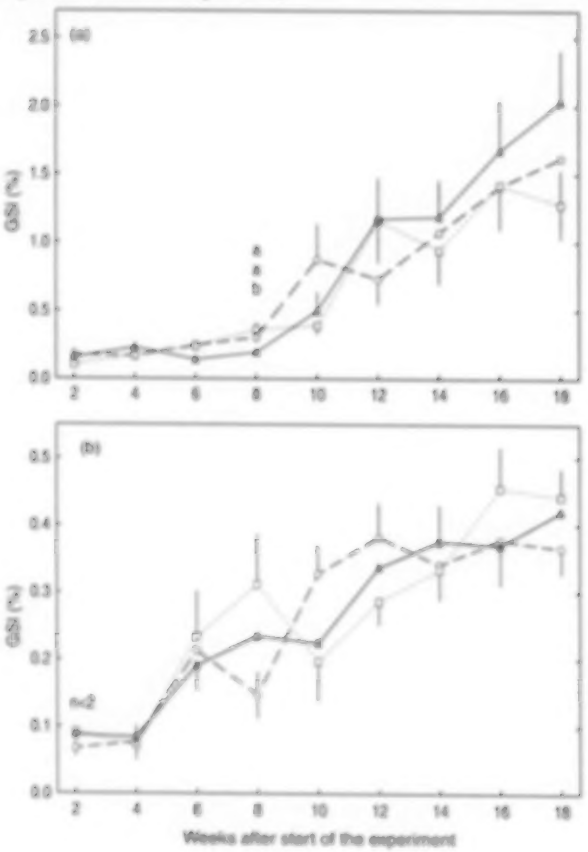
Fig. 4. Gonadosomatic index (GSI) of (*a*) females and (*b*) males of *Oreochromis niloticus* under three oxygen concentrations: high, triangles; medium, squares; low, circles. The vertical lines indicating standard error (SE) may be obscured by symbol. Different letters arranged according to highest data point indicate statistical significant differences ($p < 0.05$).



Interestingly, the discrepancy in growth between oxygen groups occurred later in males than in females, and differences between sexes occurred latest in the high oxygen group. One explanation might be that males tolerate hypoxic conditions better than females, as shown by Babiker (1984), and this would also agree with the different patterns observed between large and small water bodies (see above).

Condition factor

An increase in condition factor with increasing size was observed in all groups. The condition factor is associated with energy reserves in fish (Herbinger and Friars 1992), and although significant differences between the groups were found only temporarily, the lowest condition factor was found in the low oxygen group, which had higher ventilation frequency and lower feeding rate. Brett (1979) suggested that reduced oxygen content could act as a cue for reduction of appetite, and Fernandes and Rantin (1994) showed that for *O. niloticus*, 20% of the total oxygen uptake is required for increased gill ventilation when the oxygen

Fig. 5. Proportions (%) of sexual mature females of *Oreochromis niloticus* under three oxygen concentrations (high, triangles; medium, squares; low, circles) as a function of age. A logistic regression shows the relationship between the relative number of sexually mature and immature females. The logistic regression functions for each oxygen group are as follows: $y(\text{high}) = (1 + \exp(6.330 - 0.636 \cdot x)^{-1})100$; $y(\text{medium}) = (1 + \exp(6.584 - 0.708 \cdot x)^{-1})100$; $y(\text{low}) = (1 + \exp(5.069 - 0.523 \cdot x)^{-1})100$.

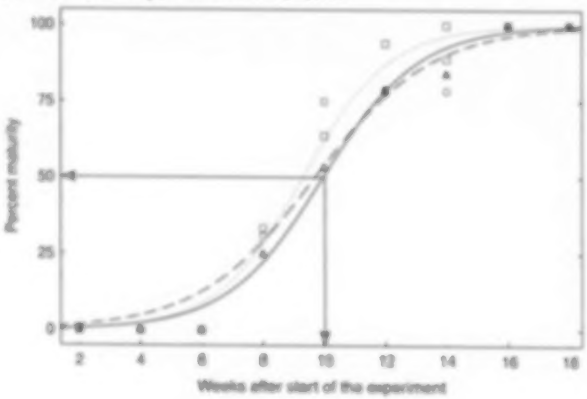
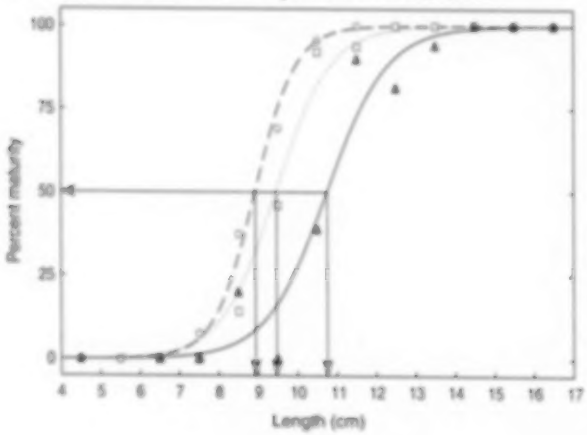


Fig. 6. The percentage of sexually mature female of *Oreochromis niloticus* under three oxygen concentrations (high, triangles; medium, squares; low, circles) as a function of size (length class 1 cm). A logistic regression shows the relationship between the relative number of sexually mature and immature females. The L_{50} for each oxygen group is shown with arrows. The logistic regression functions for each oxygen group areas follows: $y(\text{high}) = (1 + \exp(13.911 - 1.295 \cdot x)^{-1})100$; $y(\text{medium}) = (1 + \exp(13.551 - 1.435 \cdot x)^{-1})100$; $y(\text{low}) = (1 + \exp(17.096 - 1.917 \cdot x)^{-1})100$.



concentration decreases to 25% of normal levels. That the maximum rate of food intake is related to the capacity of oxygen uptake has been shown experimentally (Brett 1979; Tsadik and Kutty 1987) and in a simulation model (van Dam and Pauly 1995). Thus, for heavily ventilating, stressed fish in a low oxygen environment, everything else being equal, the maintenance metabolism will be higher and growth will be lower, which should induce reduced size at maturity according to Pauly's theory.

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Table 6. Body weight, body length, gonadosomatic index (GSI), egg size, absolute fecundity, and relative fecundity of ripe females reared under three ambient oxygen concentrations (high, medium, and low).

Parameters	High	Medium	Low
Fish weight (g)	64.8±10.55a	51.7±14.01b	34.6±9.59c
Fish length (cm)	15.0±0.83a	13.9±1.09b	12.4±1.11c
GSI (%)	3.89±0.60a	3.57±0.53ab	3.09±0.54b
Egg size (mm)	2.30±0.097a	2.23±0.097ab	2.17±0.109b
Absolute fecundity	595±171a	462±169a	271±85b
Relative fecundity	9.1±1.9a	8.8±1.4a	8.0±2.3a

Note: Results are given as mean ± standard deviation (SD). Values not followed by the same letter are statistically different ($p < 0.05$). Numbers of observations (n) in the high, medium, and low oxygen groups are 12, 7, and 12, respectively.

Table 7. Summary of the different hypotheses tested, previous predictions if oxygen is the causing agent of the stunting phenomenon, and the results obtained in the present experiment.

Hypothesis tested	Previous prediction	Result
H_1: low oxygen concentration suppresses the growth and induces stunting	Inhibited somatic growth (Pauly 1981; van Dam and Pauly 1995)	Yes
	Higher initial growth rate (Iles 1973)	No
H_2: low oxygen concentration triggers early sexual maturation at a smaller size	Sexual maturation at smaller size (Pauly 1984; Kolding 1993; Brummet 1995)	Yes
	Sexual maturation at earlier age (Kolding 1993; Brummet 1995)	No
H_3: low oxygen concentration decreases the size of the eggs but increases the relative fecundity	Smaller eggs (Peters 1963; Iles 1973; Lowe-McConnell 1982)	Yes
	Higher relative fecundity (Peters 1963; Iles 1973; Lowe-McConnell 1982)	No

Size and age at sexual maturation

Significant differences in GSI were not found between the different oxygen groups, except for females at week 8, or in Age_m for sexually mature females. However, at Age_m, the low and medium oxygen groups matured at a significantly smaller length (L_m) than the high oxygen group. The observed differences in size at maturity between the oxygen groups support Pauly's hypothesis. The observed constant Age_m, however, seems to be in disagreement with some of the definitions of stunting, where Age_m is expected to decrease (Lowe-McConnell 1958; Iles 1973; Noakes and Balon 1982). A constant age at maturity is compatible with Pauly's hypothesis, as the underlying principle relies on an approximately constant L_m–L_∞ ratio under different conditions and the different groups appeared to approach L_∞ in the same fashion. Our observations that the fastest-growing fish matured first in the different oxygen groups is also in accordance with Pauly's hypothesis as fish with faster growth reach the L_m–L_∞ ratio earlier than slower growing fish, everything else being equal.

Phenotypic plasticity and reaction norms

It is recurrently debated whether fish mature at a fixed size or a fixed age and whether age and size at maturity are genetically or environmentally determined (Policansky 1983; Stearns and Crandall 1984). Although it may appear that sexual maturation is mainly age-determined in the present experiment, both feeding experiments with *O. niloticus* (Wee and Tuan 1988; Siddiqui et al. 1997) and observations from wild populations (Duponchelle and Panfili 1998) have shown that age at sexual maturation may vary. Gunasekera et al. (1995) showed that sexual maturation was dependent on size, whereas Kronert et al. (1989) and Oldorf et al. (1989) showed that it is possible to alter the age at maturity by genetic selection. From a life-history point of view, the benefit of early maturation is that organisms have a higher probability of surviving to maturity (Iles 1973; Bell 1980). However, the benefit of early maturation depends on local conditions and selective factors such as juvenile or adult mortality, and growth rate might have an influence on this trait (Stearns 1992; Kolding 1993; Duponchelle and Panfili 1998).

This discussion ultimately leads to the use of reaction norms, which have been applied to explain the phenotypic plasticity in size and age at maturity (Stearns 1992). Several authors (Fryer and Iles 1969; Iles 1973; Noakes and Balon 1982) have proposed that stunted tilapia have a higher initial growth rate than nonstunted fish, resulting in intersecting growth curves. However, the results of the present experiment showed reduced growth rates of the stunted fish and nonintersecting growth curves of the three oxygen groups. There seems to be no empirical evidence of intersecting growth curves for the same genetic strain, and the notion of accelerated growth (often misinterpreted from higher K values of stunted fish) may therefore simply be a size-dependent artefact, as illustrated by the SGR values. The present results further question the generally assumed phenotypic plasticity of age at first maturity (Age_m) of tilapias, suggesting that it is length at first maturity (L_m), instead, which varies with different environmental conditions. Accurate age determination of wild fish in tropical areas is often a problem (Lowe-McConnell 1958; Fryer and Iles 1972; Sparre and Venema 1998). Accordingly, therefore, a small mature fish may incorrectly be considered younger than it actually is. Most of the previous work on tilapia in the wild is based on either growth or

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size at maturity (actually, breeding size) (Lowe-McConnell 1958; El Zarka et al. 1970*a*; James and Bruton 1992). Only few investigations give data on both age and length at maturity (El-Zarka et al. 1970*b*; Babiker and Ibrahim 1979). The results of this experiment, with similar age at sexual maturation but different growth rates, appears to be in accordance with the upper part of an L-shaped trajectory, as proposed by Stearns and Crandall (1984).

Reproductive traits

For the mature females, positive relationships between GSI, egg size, body size, absolute fecundity, and oxygen concentration were found. The increase in egg size and absolute fecundity with increasing fish size is in accordance with some observations on tilapia (Peters 1963; Legendre and Ecoutin 1989), whereas others have shown that small fish might produce larger amounts of small eggs to compensate for the reduction in size (Wee and Tuan 1988). The literature on GSI is also inconsistent. In contrast to our results, higher GSI has been observed in smaller individuals of *Oreochromis mossambicus* (Blühdorn and Arthington 1990) and GSI was suggested as an indicator of stunting, whereas Eyeson (1983) found no correlation between the GSI and fish size in pond-reared blackchin tilapia, *Sarotherodon melanotheron*. When the results from the present study are taken together with previously published results, GSI does not appear to be a reliable indicator of stunting in tilapia.

Although no spawning was observed in this experiment, the results indicate no direct advantage of stunting from a life-history point of view, as suggested by Iles (1973), as both smaller and fewer eggs were produced. There may, however, be possible indirect advantages from the ability of stunted populations to colonize oxygen-deficient habitats where reduced interspecific competition for food and diminished predation occur (Junk et al. 1983; Kolding 1993; Chapman et al. 1995).

A summary is presented of the different hypotheses investigated, predictions from previous studies, and the results obtained from the present experiment (Table 7). The results demonstrate that ambient oxygen can cause phenotypic variability, and that low oxygen concentrations can reduce somatic growth and size at first maturation in *O. niloticus*. The change in growth rates with size followed the same pattern for all groups, but with different absolute levels corresponding to oxygen concentration. Interestingly, the males seemed less affected than the females. The observed size at maturity and the estimated ultimate size followed the oxygen concentration, as predicted by Pauly's (1984) hypothesis. On the other hand, all groups matured simultaneously after 18 weeks, in contrast to what may be expected from the conventional definitions of stunting. If oxygen is the main trigger for stunting in tilapia, then these findings challenge the assumed plasticity in age at first maturity. Similarly, stunting does not seem to represent a unique recruitment mechanism linked to increased reproductive effort, as suggested by Iles (1973), as the smaller fish in the low oxygen group produced both smaller and fewer eggs than the larger fish in the high oxygen group. The present results, however, suggest that evolutionary success of tilapias simply could be due to their unique ability to withstand hypoxic conditions.

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Year-class strength and growth rates after metamorphosis of Japanese sardine (*Sardinops melanostictus*) in the western North Pacific Ocean during 1996–2003

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Abstract: Growth trajectories during larval to early juvenile stages in Japanese sardine (*Sardinops melanostictus*) were backcalculated based on the widths of otolith daily increments from 1996 to 2003 in the nursery grounds, Kuroshio–Oyashio transitional waters. Planktonic larvae hatched near Kuroshio have been transported northeastward from the Kuroshio waters to the transitional waters. We show that the somatic growth rates sharply increased after metamorphosis to the early juvenile stage, whereas previously, others showed that growth rate decreases during the larval stage. Otolith growth rates were more variable during the juvenile stage than the larval stage. Interannual variations in the otolith growth rate after metamorphosis explained more than 80% of variation in abundances of recruits (ca. 8–10 months old) in the Pacific coastal waters, whereas no correlation was found between the growth rate during the early larval stage and abundance. Our result was consistent with the hypothesis of growth rate-dependent recruitment success in the Kuroshio–Oyashio transitional waters.

Résumé : Nous avons déterminé par rétrocalcul les trajectoires de croissance depuis la larve jusqu'au début du stade juvénile chez la sardine japonaise (*Sardinops melanostictus*) d'après les largeurs des incréments journaliers des otolithes de 1996 à 2003 sur les nourriceries, les eaux de transition Kuroshio–Oyashio. Les larves planctoniques écloses près de Kuroshio sont transportées vers le nord-est, des eaux de Kuroshio vers les eaux de transition. Les taux de croissance somatique augmentent de façon nette après la métamorphose au premier stade juvénile; d'autres ont montré antérieurement une réduction du taux de croissance au cours du stade larvaire. Les taux de croissance des otolithes sont plus variables durant le stade juvénile que durant le stade larvaire. Les variations interannuelles du taux de croissance des otolithes après la métamorphose expliquent plus de 80 % de la variation d'abondance des recrues (âgées d'environ 8–10 mois) dans les eaux côtières du Pacifique, alors qu'il n'existe pas de corrélation entre le taux de croissance au début de la période larvaire et l'abondance. Nos résultats sont compatibles avec l'hypothèse qui veut que le succès du recrutement dans les eaux de transition de Kuroshio-Oyashio dépende du taux de croissance.

[Traduit par la Rédaction]

Introduction

Successive failures of recruitment to adult stock caused a dramatic decline in the abundance of Japanese sardine (*Sardinops melanostictus*) in the late 1980s to the early 1990s. Abundance of *S. melanostictus* in the Pacific coastal waters off Japan attained 550 billion fish in 1986, thereafter dramatically decreasing to 3 billion fish in the early 2000s (Nishida et al. 2005). The decrease of the population has been caused by the collapse of the population in the Doto region off eastern Hokkaido, the northernmost fishing grounds for sardine (Fig. 1*a*; Wada 1988; Kuroda 1991). The proportion of age-1 recruits to the total population has constantly decreased since 1986 in the Doto region, and older fish constituted most of fishery catch in the 1990s (Watanabe et al. 1995).

Spawning of *S. melanostictus* occurred in the Pacific coastal waters on the continental shelf at depths shallower than 200 m off southern and central Japan in the late 1970s; however, the center of spawning area eventually moved southward to the offshore waters over the axis of the Kuroshio in the late 1980s when the sardine population attained its maximum size (Watanabe et al. 1996; Zenitani 2001). The spawning area in the late 1990s and 2000s was located close to the coastal area again (Kubota 1998, 2004). Many studies, therefore, have been conducted on inshore–offshore differences in growth and survival during the early larval stages in the Kuroshio waters. Watanabe et al. (1996) esti-

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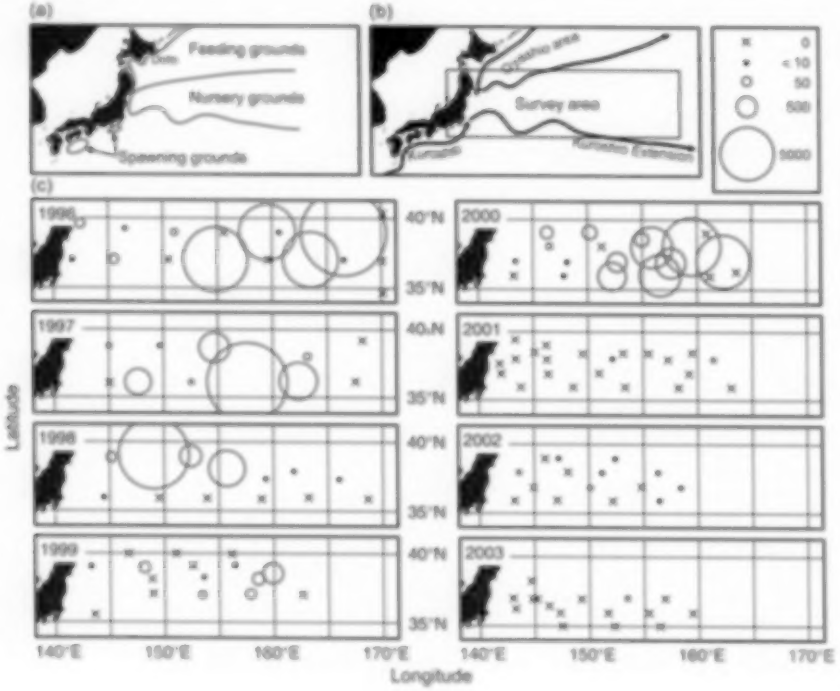
Fig. 1. Ecological and oceanographic diagrams for *Sardinops melanostictus* in the Pacific waters off Japan: (*a*) schematics of locations for spawning, nursery, and feeding grounds for *S. melanostictus*; (*b*) schematics of current structures (thick lines and arrows) around the survey area (open box); (*c*) distribution density of late larval and early juvenile *S. melanostictus* during 1996–2003 in the Kuroshio–Oyashio transitional waters. Crosses (×) and open circles indicate total number of individual fish collected in three trawl hauls at a night. The scales are shown in the right uppermost panel.



mated survival rates during ~1 week after hatching based on abundances of eggs and planktonic larvae during 1978–1992 and found that the rates were greater in the Kuroshio axis than in the coastal waters in the late 1980s. From the viewpoint of the nutritional conditions, Kimura et al. (1996) examined the RNA–DNA ratio of larvae collected from the Kuroshio waters and showed that almost all larvae were in good health. Production of small copepods including nauplii was sufficient for the food requirement of sardine larvae in the Kuroshio frontal region (Nakata et al. 1995).

Abundances of neither egg nor larva (~1 week old) in the Kuroshio waters, however, correlated with abundances in age-1 recruits in the Doto region during 1978–1992 (Watanabe et al. 1995). Heath et al. (1998) demonstrated, based on the flow field of the Kuroshio, that the eggs and newly hatched larvae distributed near the Kuroshio axis were transported northeastward into the Kuroshio Extension waters within 2 weeks. Subtle changes in growth and development rates during early life stages in fishes can cause large differences in recruitment to the adult stock (Houde 1987; Anderson 1988; Houde 1989). Hence, growth and development rates through the late larva and juvenile stages in the waters between the Kuroshio Extension and the Doto region, which corresponds to the Kuroshio–Oyashio transitional waters, have been hypothesized to be key factors for recruitment (Watanabe et al. 1995; Watanabe 2002).

Width of otolith increments were positively correlated with somatic growth rates during late larval and early juvenile stages for Japanese anchovy (*Engraulis japonicus*) in the experimental studies (Takahashi and Watanabe 2004*a*). Larvae with faster growth rates had a higher probability of survival to the adult stage than larvae with slower growth rates in the Kuroshio–Oyashio transitional waters (Takahashi and Watanabe 2004*a*). The widths of otolith increments in juvenile *S. melanostictus* could allow us to estimate growth rate from hatch in the Kursohio waters to catch in the Kuroshio–Oyashio transitional waters.

Here we hypothesize that recruitment success of *S. melanostictus* in the Kuroshio–Oyashio transitional waters is related to growth rate (Watanabe 2002). We reconstruct growth trajectories based on the width of otolith increments from hatch to catch of *S. melanostictus* juveniles collected in the trawl survey for forecasting recruitment abundance of sardine in the Kuroshio–Oyashio transitional waters from 1996 to 2003. Variations in the abundance of recruits in the Pacific coastal waters were examined in relation to the year-to-year differences in growth. Reconstructed growth trajectories from the spawning grounds in Kuroshio waters to the nursery grounds in Kuroshio–Oyashio transitional waters could elucidate the developmental stage that is critical for growth-selective survival and, thus, account for that variation in the abundance of recruits.

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Table 1. Collection data for late larval and juvenile *Sardinops melanostictus* in the Kuroshio–Oyashio transitional waters.

Year	Date	No. of trawl haul	No. of fish	
			Total	Growth analysis
1996	6 June – 26 June	46	13 879	174
1997	24 May – 25 June	31	5 707	146
1998	14 May – 14 June	37	4 293	127
1999	25 May – 15 June	44	464	95
2000	15 May – 8 June	58	8 161	342
2001	12 May – 6 June	63	11	10
2002	10 May – 28 May	58	40	18
2003	11 May – 28 May	47	10	5

Materials and methods

Sample collection

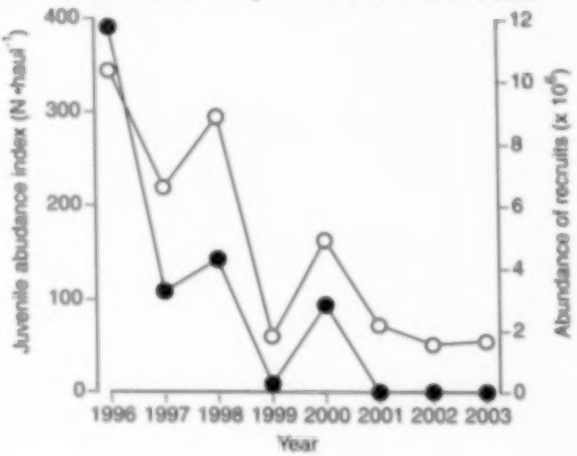
Samples of late larvae and juveniles of *S. melanostictus* in the Kuroshio–Oyashio transitional waters were obtained in the subsurface trawl survey conducted by the National Research Institute of Fisheries Science in May and June during 1996–2003 (Table 1; Fig. 1). The trawl net has an opening of 25 m × 25 m and a cod end of 10 mm in mesh aperture and was towed for 30 min with a ship speed of 3–4 knots. Three trawl hauls per night were made in the stratum shallower than 25 m. Late larval and juvenile *S. melanostictus* were sorted from the trawl catch at each station and subsamples of up to 100 individuals were selected randomly at the positive stations. Captured larvae and juveniles were preserved by freezing at –25 °C.

Growth analysis

Each fish was measured, with digital calipers, for standard length (SL) to the nearest 0.1 mm. Sagittal otoliths of larval and juvenile *S. melanostictus* were dissected out, cleaned under a dissecting microscope at 10–20× magnification, and mounted on a glass slide with enamel resin. Otoliths of fish larger than ~40 mm SL were ground with sandpaper (no. 2000) and smoothed with a lapping film (no. 8000). A random sample of up to 30 otoliths taken at each trawl station was used for the growth analysis. Otolith radius (OR) from the nucleus to the margin and increment width (I) were measured along a transect on the postrostrum of the otolith. The measurement was conducted using an otolith measurement system (Ratoc System Engineering Co. Ltd., Tokyo, Japan) consisting of a light microscope with 100–500× magnification, a CCD camera, a video monitor, and an image analyzer controlled by a personal computer.

Otolith increments were formed on a daily basis, with the first daily increment formed 2–3 days after hatching, corresponding to the onset of first feeding in *S. melanostictus* (Hayashi et al. 1989). Hayashi et al. (1989) incubated field-collected eggs, reared hatched larvae at ~18 °C for 30 days, took larval samples every 5 days, and counted the number of otolith increments with light and scanning electric microscopes. The daily age at capture of each larva and juvenile was calculated by adding two to the total number of otolith daily increments. SL at ages through the larval and the juvenile stages was backcalculated for each individual based on

Fig. 2. Interannual variation in the juvenile abundance index (solid circle) calculated from temperature-specific density of late larval and early juvenile *Sardinops melanostictus* in the Kuroshio–Oyashio transitional waters and abundance of recruits (open circle) calculated based on VPA analysis in the Pacific coastal waters.



the relationship between SL and OR using the biological intercept method (Campana 1990; Campana and Jones 1992). The SL–OR relationship was linear in this study, whereas the relationship during the larval stage has been reported to be logarithmic (Oozeki and Zenitani 1996) or allometric (Watanabe and Kuroki 1997). Because SL at the inflection point between the linear equation and the allometric/logarithmic equations was ~25 mm (see Results), we assumed that the SL–OR relationship changes at ~25 mm SL during the development of *S. melanostictus*. Linear parameters of the SL–OR relationship from hatching to catch were approximated for each fish as follows:

$$(1) \qquad a = (L_3 - L_c)/(R_3 - R_c)$$

$$(2) \qquad b = L_c - a \cdot R_c$$

where L_3 is SL at the deposition of the first increment corresponding to age 3 days, R_3 is radius from the center of the nucleus to the first thick increment, L_c and R_c are SL and OR at capture, respectively. Live total length (TL) at first feeding for *S. elanostictus* was ~6.0 mm at 17.8 °C in the captive study (Matsuoka and Mitani 1989). Because the relationship between SL and TL for newly hatched larvae to 80 mm juveniles was represented by a linear equation (SL = 0.84TL + 0.89, R^2 = 0.999), live SL at the deposition of the first increment was fixed at 5.9 mm in this study. SL at age n days was calculated for each fish using the linear equation $L_n = aR_n + b$ (n = 3, 4, …, c–1, c (capture)) and excluded age range with backcalculated SLs ≤ 25 mm because there may be error due to a nonlinear relationship between SL and OR.

To estimate growth trajectories from the spawning grounds to the nursery grounds, the mean increment width during the 10-day intervals between hatching and capture was calculated for each fish as

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Fig. 3. Frequency distribution in standard length (SL) of late larval and early juvenile *Sardinops melanostictus* collected from the Kuroshio–Oyashio transitional waters during 1996–2003.

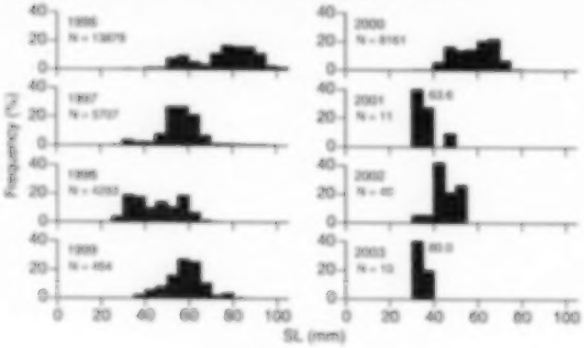
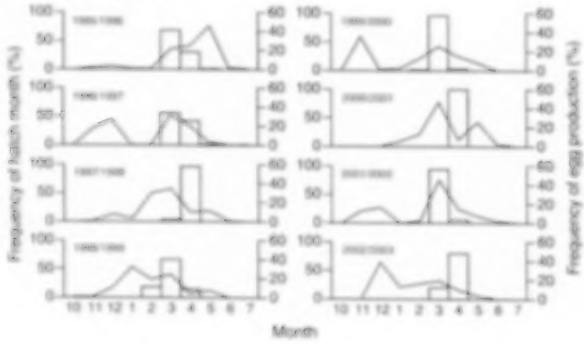


Fig. 4. Frequency distribution of hatch dates (open bars) of late larval and early juvenile *Sardinops melanostictus* collected in the Kuroshio–Oyashio transitional waters and egg distributions (solid line) in the Kuroshio waters. Note that numbers on *x* axis indicate the months of the year.



$$(3) \qquad I_{10i} = \frac{R_{10i} - R_{10(i-1)}}{10}$$

where R_{10i} and $R_{10(i-1)}$ are increment radii at ages $10i$ and $10(i - 1)$ days ($i = 2, 3, \ldots, 6, 7$). Because the first daily increment corresponds to age 3 days, I_{10} ($i = 1$) was calculated exceptionally as $I_{10} = (R_{10} - R_3)/7$.

Because most of the late larvae and early juveniles reached 25 mm SL by age 40 days, the mean growth rate during 10-day intervals was calculated for each fish as

$$(4) \qquad G_{10j} = \frac{L_{10j} - L_{10(j-1)}}{10}$$

where L_{10j} and $L_{10(j-1)}$ are backcalculated lengths at ages $10j$ and $10(j - 1)$ days ($j = 4, 5, 6, 7$).

To reconstruct actual growth trajectories as year classes, mean I in 10-day intervals at each trawl station ($\bar{I}$) were weighted by individual number of collected fish as

$$(5) \qquad \bar{I} = \sum I \cdot n/N$$

where n is the number of individual fish collected at the trawl station and N is the total number of individual fish per year. The weighted mean G in each trawl station also was weighted by individual number of collected fish substituting G for I in eq. 5.

Fig. 5. Relationship between standard length (SL) and otolith radius (OR) at catch (open circles) of late larval and early juvenile *Sardinops melanostictus* from 1996 to 2000. Solid and dotted lines indicate the SL–OR relationship reported in Oozeki and Zenitani (1996) and Watanabe and Kuroki (1997), respectively.

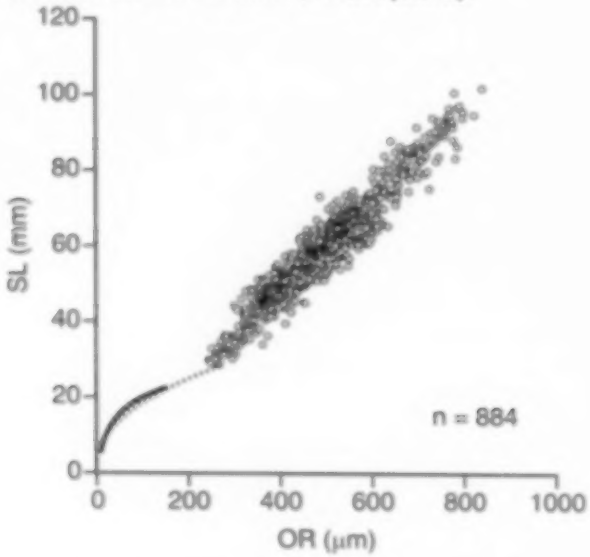


Table 2. Regressional equations for relationship between standard length (SL) and otolith radius (OR) of the late larval and juvenile *Sardinops melanostictus*.

Year	*N*	Linear equations	R^2
1996	174	SL = 0.120OR + 0.27	0.962
1997	146	SL = 0.112OR + 4.65	0.920
1998	127	SL = 0.108OR + 2.48	0.938
1999	95	SL = 0.095OR + 11.0	0.857
2000	342	SL = 0.109OR + 7.43	0.878
Total	884	SL = 0.116OR + 2.31	0.926

Data on year-class strength and egg production

Abundance of adult *S. melanostictus* in the Pacific coastal waters has been estimated since 1976 by National Research Institute of Fisheries Science (Yokohama, Kanagawa, Japan). Individual number of age-0 recruits of size 10–15 cm in Pacific coastal waters was calculated by virtual population analysis (ADAPT-VPA; Pope 1972), based on time series of catch-at-age data, and tuned with the distribution density of the larval and juvenile *S. melanostictus* in this survey (Nishida et al. 2005).

To know egg production of small pelagic fishes, including *S. melanostictus*, an egg and larval census has been conducted using ring net in the Pacific coastal waters off southern and central Japan since 1978. Hatch dates of the late larvae and early juveniles in the Kuroshio–Oyashio transitional waters were compared with the monthly egg production in the Pacific coastal waters off southern and central

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Table 3. Backcalculated length at 40, 50, 60, and 70 days of late larval and juvenile *Sardinops melanostictus* in the Kuroshio–Oyshio transitional waters.

	Length at 40 days		Length at 50 days		Length at 60 days		Length at 70 days	
Year	*N*	Mean ± SD	*N*	Mean ± SD	*N*	Mean ± SD	*N*	Mean ± SD
1996	169	31.1±3.4a	165	42.4±4.8a	133	53.2±5.3a	106	64.9±7.0a
1997	131	31.5±3.5a	131	40.9±6.3a	74	50.0±7.8b	35	58.9±10.1b
1998	88	28.1±2.1b	115	35.7±4.5c	81	46.7±5.8c	22	53.8±8.8c
1999	59	29.5±3.2b	91	35.9±6.3c	86	44.3±8.2c	61	51.4±9.7c
2000	288	29.2±2.7b	337	38.1±4.7b	277	48.1±5.7b,c	188	58.5±7.1b
2001	7	30.0±2.2b	4	36.2±6.6a,b,c				
2002	14	29.5±2.4b	18	38.7±4.1a,b,c	5	45.0±3.3a,b,c		
2003	4	28.9±3.7b	1	31.6				

Note: *N*, individual number for statistical test; SD, standard deviation. Different letters following values indicate significant differences ($p < 0.05$).

Japan (Kimura 1996; Kubota 1998, 2004; Ishida 1999, 2001).

Statistical analysis

Differences in slopes and intercept of the relationship between SL and OR were tested by applying analysis of covariance (ANCOVA). Differences in backcalculated SL were tested for using analysis of variance (ANOVA) and post hoc test Scheffé's *F*. Standardized anomalies from the mean *G* and *I* for each 10-day interval were related to standardized anomalies from the number of age-0 recruits ($\log_{10}$-transformed, hereafter called the anomaly of logarithmic abundance) using a linear regression analysis. Standardized anomaly (*A*) is considered to reflect variations from the mean and was calculated based on the following equation:

$$A = (x - \bar{x})/\mathrm{SD}, \quad (6)$$

where x and $\bar{x}$ represent a value and the mean of values, respectively, and SD, indicates standard deviation.

Results

Abundance

Late larval and juvenile *S. melanostictus* were more abundant during 1996–2000 in the northern or eastern waters than in the southern or western waters of the survey area (Fig. 1). The more than 100 fish per haul were caught in 1996, 1997, 1998, and 2000, whereas less than 10 fish per haul were caught in 1999, 2001, 2002, and 2003. The estimated number of age-0 fish decreased from 10.3 billion in 1996 to 1.8 billion in 1999 and increased to 4.9 billion in 2000, whereas after 2001, it ranged from 1.6 to 2.2 billion (Fig. 2).

Body length

SL of the collected fish ranged from 27.0 to 102.0 mm in the eight survey years (Fig. 3). Modes of the SL distributions were within the range of 45–60 mm in 1996–2000, 75–80 mm in 1996, 30–35 mm in 1998, and 65–70 mm in 2000. SL ranged from 32.6 to 53.6 mm in 2001, 2002, and 2003, corresponding to a smaller range than in the first five survey years.

Hatch dates and spawning

Hatch dates of the collected fish ranged from 10 February to 3 May (Fig. 4). Proportion of fish hatched in March and April to total number of fish ranged from 85% to 100% in the eight survey years. Proportions of fish hatched in February were 18.4% in 1999 and 0.8% in 2000, and those in May were 0.7% in 1996 and 1.8% in 1997. Eggs of *S. melanostictus* were collected from November to June in the Kuroshio waters. Hatch dates for the late larvae and early juveniles in the Kuroshio–Oyashio transitional waters corresponded to late winter to early spring of the spawning season in the Kuroshio waters.

Somatic and otolith growth

SL is shown as a function of OR for all data for 1996–2000 (Fig. 5). The SL and OR relationships are represented by linear equations (Table 2). The slopes of the equations are significantly different among five survey years (ANCOVA, $P < 0.0001$). The slopes are smallest in 1999 and largest in 1996. Because there were less than 20 specimens in 2001–2003, they were excluded from the analysis of the SL and OR relationship. Inflection points in the SL–OR relationship between the linear equations in this study and the allometric–logarithmic equations, which were previously reported in the SL range from 5 to 30 mm, were 24.1 mm SL and 188 μm OR for Oozeki and Zenitani (1996) and 24.9 mm SL and 195 μm OR for Watanabe and Kuroki (1997).

Growth rates through larval to juvenile stages

Mean backcalculated SL increased from 31.1 ± 3.4 mm at age 40 days to 64.9 ± 7.0 mm at age 70 days in 1996 and from 29.5 ± 3.2 mm at age 40 days to 51.4 ± 9.7 mm at age 70 days in 1999 (Table 3). The SL increased linearly as a function of age in 1996 and 1997, but asymptotically in 1998, 1999, and 2000 (Fig. 6). Because only one fish was >50 days in 2003, backcalculated SL at age 50 days in 2003 was excluded from the statistical analysis.

The weighted mean width of otolith increments in 10-day intervals increased with increasing age in all eight survey years (Fig. 7). I_{10} in 1996 was 2.7 μm and similar to that in 1999 (2.8 μm), whereas I_{70} in 1996 was 9.7 μm and wider than that in 1999 (7.8 μm). The weighted mean growth rates in 10-day intervals also increased similar to *I*. The G_{50}, G_{60}, and G_{70} were faster in the first half of the survey period (1996–1998) than in the second half (1999–2003), except for 2000.

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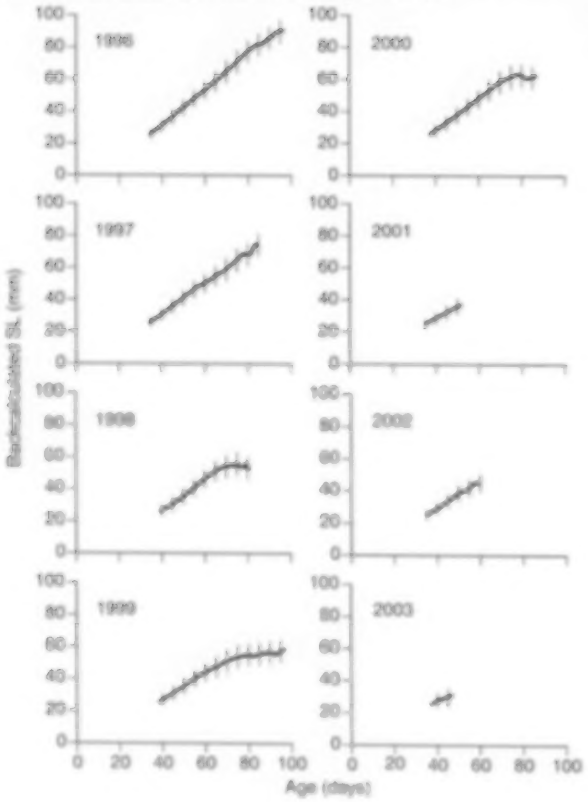
Fig. 6. Backcalculated standard length (SL) in relation to daily age of late larval and early juvenile *Sardinops melanostictus* in the Kuroshio–Oyashio transitional waters during 1996–2003. Vertical bars indicate standard deviations at 5-day intervals.



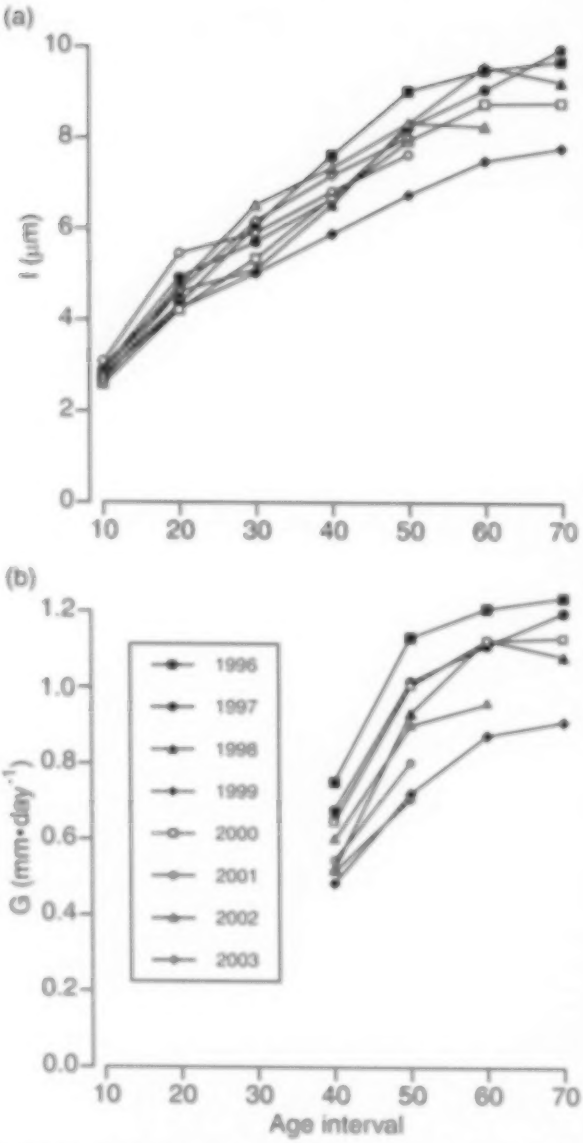
Year-class strength and growth rates

Standardized anomalies for logarithmic abundance of age-0 fish positively correlated with the anomalies for I_{60} and I_{70}, whereas no significant relationship was found through I_{10} to I_{50} (Fig. 8). The positive relationship between the standardized anomaly of logarithmic abundance (Y) and I_{60} and I_{70} were represented by the following linear equations: $Y = 0.94I_{60} + 0.27$ ($N = 6$, $R^2 = 0.86$, $P = 0.0083$) and $Y = 0.77I_{70} + 0.54$ ($N = 5$, $R^2 = 0.78$, $P = 0.047$), respectively.

The standardized anomalies for the logarithmic abundance of age-0 fish were positively correlated with the anomalies for G_{50} and G_{60}, whereas no significant relationship was found in G_{40} and G_{70} (Fig. 9). The positive relationship between the standardized anomaly of logarithmic abundance and G_{50} and G_{60} were represented by the following linear equations: $Y = 0.83G_{50} + 0.0015$ ($N = 8$, $R^2 = 0.69$, $P = 0.010$) and $Y = 0.95G_{60} + 0.27$ ($N = 6$, $R^2 = 0.88$, $P = 0.0061$), respectively. Although no significant relationship was found to G_{70}, the relationship was represented by a linear equation ($Y = 0.74G_{70} + 0.54$, $N = 5$, $R^2 = 0.74$, $P = 0.063$).

R^2 of the regressions of the logarithmic abundance of age-0 fish and I ranged from 0.01 to 0.1 for the intervals between the ages of 10, 20, 30, and 40 days and then sharply increased to 0.38 at 50 days and 0.86 at 60 days (Fig. 10). R^2 of the regression of the logarithmic abundance and G increased from 0.39 at 40 days to 0.88 at 60 days.

Fig. 7. Changes in otolith and somatic growth rates with 10-day intervals of late larval and early juvenile *Sardinops melanostictus* collected in the Kuroshio–Oyashio transitional waters during 1996–2003. (*a*) Increment width (I) in otolith. Closed (open) squares, circles, triangles, and diamonds indicate 1996 (2000), 1997 (2001), 1998 (2002), and 1999 (2003) as shown in the legend of the symbols in (*b*). (*b*) Backcalculated growth rates (G) after age interval of 40.



Discussion

Somatic and otolith growth

The slopes of the SL–OR linear equations were smallest

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Fig. 8. Relationship between standardized anomalies of logarithmic recruitment abundance and increment width (I) in 10-day intervals.

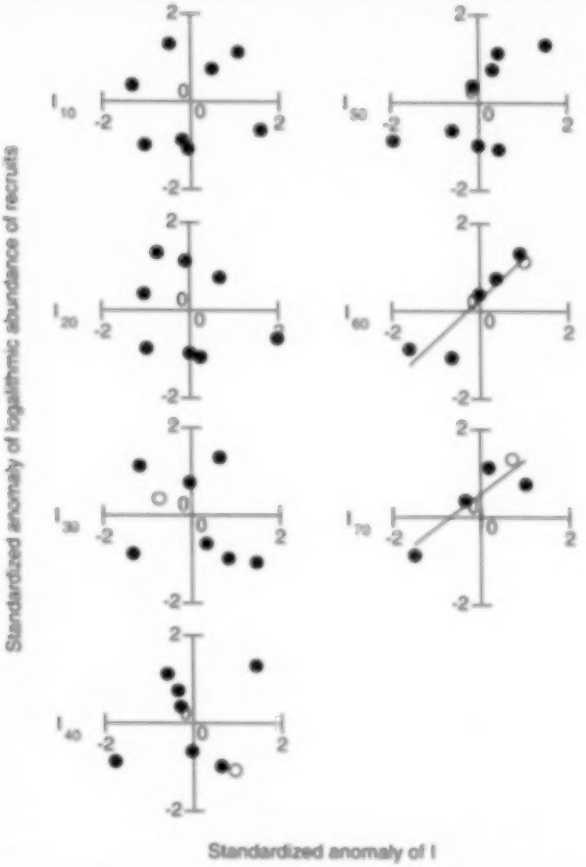
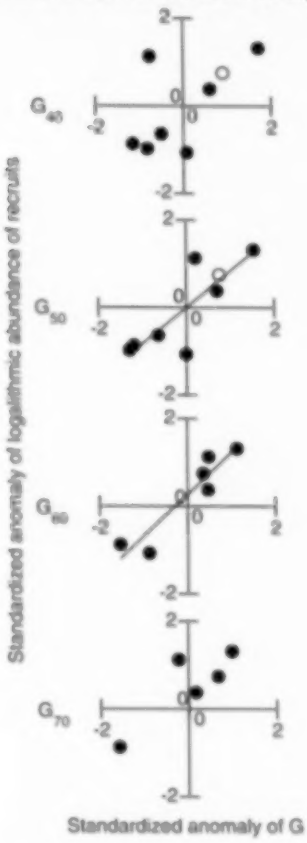


Fig. 9. Relationship between standardized anomalies of logarithmic recruitment abundance and growth rate (G) in 10-day intervals.

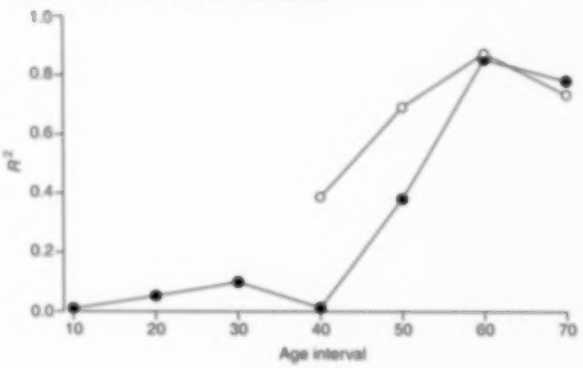


in 1999 and largest in 1996, indicating that OR at a given SL was larger in 1999 than in 1996. Mean backcalculated SLs through ages 40 to 70 days were significantly larger in 1996 than in 1999. This result indicates that otolith radius at a given body size is larger in the juveniles with slower growth rates than in those with faster growth rates. For the larval *S. melanostictus* up to ~30 mm SL, OR at a given body length was also larger in the larvae with slower growth rates than in those with faster growth rates in the Kuroshio waters (Oozeki and Zenitani 1996). Secor and Dean (1989) examined the relationship between otolith size and body length in larval and juvenile striped bass (*Morone saxatilis*) reared under various environmental conditions and demonstrated that otolith size at a given body length was consistently larger in slower-growing larvae than in faster-growing larvae.

Growth trajectories through larval to juvenile stages

The late larvae and early juveniles in the Kuroshio–Oyashio transitional waters hatched primarily in March and April. The spawning season of *S. melanostictus* ranges from November to June based on the egg production censuses in the Kuroshio waters during the survey years. *Sardinops melanostictus* eggs were collected in the coastal waters off southern and central Japan in March and April (Kubota 1998, 2004), which indicates that the late larvae and early juveniles in the transitional waters were spawned in the Kuroshio waters from late winter to spring in the recent low population period.

Fig. 10. Change in R^2 for regression equations of increment width (I; solid circles) and growth rate (G; open circles) with age intervals relative to recruitment abundance.



G sharply increased from ~0.6 mm·day^{-1} at age 40 days

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to ~1.0 mm·day^{-1} at age 60 days in the Kuroshio–Oyashio transitional waters. Meanwhile, growth rates of *S. melanostictus* decreased from ~1.0 mm·day^{-1} in the early larval stage to ~0.6 mm·day^{-1} in the late larval stage in the Kuroshio waters (Oozeki and Zenitani 1996; Watanabe and Saito 1998). In the captive study, growth rates of *S. melanostictus* decreased during the larval stage from hatch to ages 30–50 days and increased after metamorphosis (Nakamura et al. 1991). This implies that growth rates of *S. melanostictus* decrease during the larval stage up to ~30 mm SL in the Kuroshio waters and increase after metamorphosis to the juvenile stage in the transitional waters. Growth rates of Pacific sardine (*Sardinops sagax*) also decreased during the larval stage and increased after metamorphosis in the California Current region (Butler 1987). Growth change with size or ontogeny could be an inherent character in *Sadinops*.

During northeastward transport from the spawning grounds to the transitional waters, the growth rate of *S. melanostictus* is believed to be more variable in the Kuroshio–Oyashio transitional waters than in the Kuroshio waters. Pacific saury (*Cololabis saira*) spawns in the Kuroshio waters in winter and in the Kuroshio–Oyashio transitional waters in spring and fall. Watanabe et al. (2003) found that the larvae hatched in the transitional waters had greater variations in growth rate up to 40 mm than in Kuroshio waters and suggested that variability in water temperature might account for the differences in growth rates. Abundance of copepods as prey item for *C. saira* was also more variable in the transitional waters than in the Kuroshio waters (Odate 1994).

Growth-dependent recruitment

Our results indicate that interannual variations in the growth rate after metamorphosis in the transitional waters positively correlated with variations in abundance of the age-0 recruits in the Pacific coastal waters. The growth rate was controlled by temperature and limited by available prey density during the survey period in the Kuroshio–Oyashio transitional waters (M. Takahashi, Y. Watanabe, A. Yatsu, and H. Nishida, unpublished data). Hence, we concluded that for *S. melanostictus*, abundance of recruits is function of temperature and food availability mediated through growth rates during the early juvenile stage in the transitional waters. This is consistent with the hypothesis of growth rate dependent recruitment success in the Kuroshio–Oyashio transitional waters (Watanabe 2002).

More variable temperature and prey density could enhance variations in juvenile growth and probability of recruitment success for *S. melanostictus* in the transitional waters than in the Kuroshio waters. Growth trajectories for Atlantic cod (*Gadus morhua*) showed that variability of growth rate increased during the late larval to the early juvenile stages with habitat change from the planktonic to the epibenthic life and that planktonic larvae with faster growth rate survived to the epibenthic juvenile stage in higher probability than those with slower growth rate (Meekan and Fortier 1996). Larger year classes of *G. morhua* had faster growth rates during the habitat change than smaller year classes (Campana 1996).

Engraulis japonicus also uses the Kuroshio–Oyashio transitional waters as nursery grounds, as does *S. melanostictus* (Takahashi and Watanabe 2004*b*). *Engraulis japnicus* has expanded its distribution ranges from coastal waters to the offshore Kuroshio–Oyashio transitional waters with increasing population in the late 1990s (Takahashi et al. 2001; Takahashi and Watanabe 2005). In contrast to *S. melanostictus*, the late larval and early juvenile growth rates for *E. japnicus* were faster after 1999 than during 1996–1998, because temperature and available prey density became favorable for *E. japnicus* growth after 1999 in the transitional waters (M. Takahashi, Y. Watanabe, A. Yatsu, and H. Nishida, unpublished data). The out-of-phase dynamics of populations between *S. melanostictus* and *E. japonicus* in the western North Pacific Ocean could be explained by contrasting growth and survival processes in relation to environmental conditions in the Kuroshio–Oyashio transitional waters.

Adult growth in *S. melanostictus*, on the other hand, is well known to be a negative function of population size (e.g., Nakai 1962; Wada and Kashiwai 1989; Hiyama et al. 1995). Size at age 1 year was ~16 cm during the 1980s when population level was high, increasing up to 18 cm in the late 1990s during lower population size. Food limitation has been hypothesized to cause slower growth after recruitment to the adult stage and, thus, smaller body size at age during the period of large population size in the 1930s and 1980s. Tadokoro et al. (2005) estimated biomass of the copepod *Neocalanus*, which is considered to be the main food item for adult sardine in the subarctic Oyashio waters off northern Japan, and showed that daily consumption by adult sardine ranged from 32% to 138% of the daily production of this copepod in the early 1980s. Based on results in this study, we propose that larvae spawned in the Kuroshio waters in the late 1970s and 1980s were swiftly transported to the Kuroshio–Oyashio transitional waters, grew faster under optimum temperature and sufficient food conditions, and survived better during the juvenile stage over the carrying capacity of the feeding grounds as explained by Tadokoro et al. (2005).

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Matching catches to quotas in a multispecies trawl fishery: targeting and avoidance behavior under individual transferable quotas

Trevor A. Branch and Ray Hilborn

Abstract: Optimizing yield in multispecies fisheries is only possible when fishers have a high degree of control over the species mixture in their catches, although incentives to encourage this kind of behavior are rarely in place. One exception is the British Columbia, Canada, groundfish trawl fishery, where individual transferable quotas govern total allowable catches (TACs) for 22 species, combined with 100% observer coverage and the deduction of discard mortality from quota. Despite the number of species covered, when TACs were increased for some species and reduced for others, fishers were able to adjust the species mixture in their catches. The top 34 vessels frequented a wide range (mean 38, range 20–69) of "fishing opportunities" (repeated trawls along the same track line) containing predictable species mixtures. When TACs for rougheye (*Sebastes aleutianus*), shortraker (*Sebastes borealis*), and yelloweye rockfish (*Sebastes ruberrimus*) were sharply cut, fishers avoided fishing opportunities where these species were more abundant. More generally, their choice of fishing opportunities depended on the expected multispecies composition, although other factors were probably also important.

Résumé : Il est possible d'optimiser le rendement dans les pêches multispécifiques seulement lorsque les pêcheurs ont un niveau de contrôle élevé du mélange d'espèces dans leurs récoltes, bien qu'il y ait rarement des mesures pour favoriser un tel comportement. La pêche au chalut des poissons de fond en Colombie Britannique, Canada, représente une exception, car des quotas individuels transférables contrôlent les captures totales permises (« TAC ») chez 22 espèces en combinaison avec une couverture de 100 % des observateurs et la déduction du quota de la mortalité due aux rejets à la mer. Malgré le nombre d'espèces couvertes, lorsque les TAC ont été augmentées pour certaines espèces et réduites pour d'autres, les pêcheurs ont été capables d'ajuster les mélanges d'espèces dans leurs récoltes. Les 34 principaux navires fréquentent une gamme étendue (moyenne 38, étendue 20–69) de « possibilités de pêche », des traits répétés de chalutage le long de la même ligne, contenant des mélanges prévisibles d'espèces. Lorsque les TAC pour les sébastes à œil épineux (*Sebastes aleutianus*), les sébastes boréaux (*Sebastes borealis*) et les sébastes aux yeux jaunes (*Sebastes ruberrimus*) ont été réduites de manière importante, les pêcheurs ont évité les sites de pêche dans lesquelles ces espèces étaient plus abondantes. De façon plus générale, leur choix de sites de pêche dépendait de la composition multispécifique prévue, bien que d'autres facteurs aient probablement aussi été importants.

[Traduit par la Rédaction]

Introduction

Multispecies fisheries pose particular problems for the fisheries objectives of optimizing yield and preventing overfishing. Many previous papers have concluded that in multispecies (or mixed-stock) fisheries, the total yield is maximized when some species are overfished and others are underutilized (e.g., Ricker 1958; Paulik et al. 1967; Hilborn et al. 2004). For example, Hilborn et al. (2004) modeled 12 species in the US West Coast groundfish trawl fishery and showed that maximizing the overall yield from all of these species would result in some species being overfished, whereas preventing the overfishing of any species would decrease potential yield by 90%.

This paradigm is based on the assumption that the species mixture in catches depends only on abundance and catchability and that tfishers have no control over the species mixture. But if it is possible to adjust the species mixture, then the multispecies yield could increase closer to the sum of the maximum yields of the individual species without any overfishing (Ricker 1958; Paulik et al. 1967). A close examination reveals that in most fisheries, there is some ability to target and avoid species (Beverton and Holt 1957; Campbell and Nicholl 1994; Quirijns et al. 2008). However, unless fishers are given the correct incentives, they will not change the way in which they fish (Branch et al. 2006*a*; Grafton et al. 2006).

One method of providing incentives for fishers to match catches to total allowable catches (TACs) is the implementation of individual transferable quotas (ITQs). Under ITQs, the TACs for each species are divided among participants in the fishery, and these shares can be bought, sold, and leased. To maximize revenue in a multispecies ITQ fishery, fishers need to avoid species with constraining TACs and

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target those with TACs that are undercaught, while minimizing their costs of fishing. Since ITQs were first proposed (Christy 1973; Moloney and Pearse 1979), they have been implemented in a wide variety of fisheries and their effects are well known (e.g., Copes 1986; Squires et al. 1995; National Research Council 1999). The most obvious effects are a reduction in overcapitalization, improved economic efficiency, and the consolidation of quota in the hands of more efficient operators (e.g., Crowley and Palsson 1992; Grafton 1996). Year-round fishing under ITQs often results in more careful handling of fish, improvements in product quality and form, and increased ex-vessel fish prices (Gauvin et al. 1994; Herrmann 1996; Branch 2006), although when moving from a derby-style fishery to ITQs, this price increase may come through appropriating profits from existing processors (Matulich et al. 1996; Matulich and Clark 2003). With greater economic efficiency, ITQ fisheries usually generate net economic rents above and beyond those available in other fisheries, resulting in ITQ shares becoming highly valuable assets. This characteristic of ITQs encourages stewardship, thus ITQ holders may request TAC reductions (Annala 1996; Breen and Kendrick 1997; Grafton et al. 2006), may report the illegal fishing behavior of other users (Gauvin et al. 1994), and may even fund the enforcement of regulations themselves (Grafton 1996; Yandle 2006*a*, 2006*b*). However, there are important social impacts of ITQs to be weighed against these benefits. For example, fishing communities may lose out when quota is sold to outsiders (Eythórsson 2000; Skaptadóttir 2000), employment for crew members may decrease (Copes and Charles 2004), and the high price of quota will make it too expensive for young fishers to enter ITQ fisheries (Dewees 1998; McCay et al. 1998; Bradshaw 2004).

High-grading and discarding can undermine the incentives under ITQs that encourage fishers to match catches to TACs in multispecies fisheries. High-grading is the practice of maximizing income from fixed quotas by choosing to retain only the most valuable fish of a particular species, while discarding those of lesser value (e.g., small, discolored, or damaged fish). Theoretical papers have suggested that ITQs will increase high-grading in fisheries (Copes 1986; Anderson 1994; Arnason 1994), as borne out in practice in some fisheries (Boyd and Dewees 1992; Bradshaw et al. 2000), but ITQs also encourage fishers to change their fishing patterns and gear selectivity to avoid catching low-valued fish in the first place, thus high-grading has decreased in other fisheries (Arnason 1994; McCay et al. 1995; Pascoe 1997). Discarding in multispecies fisheries can occur when the individual quota is reached for one or more species. At this juncture, fishers can lease quota to cover the overage or choose to dump the excess fish and continue fishing for the remaining species. In many ITQ fisheries, especially those with no at-sea observer coverage, this form of discarding is problematic (Fraser and Jones 1989; Annala et al. 1991; Waitt and Hartig 2000), whereas in other ITQ fisheries, discarding has decreased (Casey et al. 1995; Holland 2000), especially where at-sea observer coverage is high (Branch 2006; Branch et al. 2006*b*; Grafton et al. 2007). As reviewed by Sanchirico et al. (2006), discarding problems can be alleviated through properly functioning quota markets, rollover allowances (adjusting next year's quota to account for this year's undercatches or overcatches), deemed-value payments (paying a fine for catches in excess of quota), and retrospective balancing of quota.

In theory, in a multispecies ITQ fishery, fishers should avoid species with relatively low TACs (compared with their availability for capture) and target species with relatively high TACs by carefully choosing when, where, and how to fish. They should also trade quotas with other fishers to build up a quota portfolio that closely matches the expected mixture of species in their catches. In practice, little quantitative research on this topic has been conducted, although anecdotal reports of behavioral changes are legion, including increasing or decreasing the length of trawls, avoiding high densities of species, experimenting with gear modifications, employing test trawls before fishing, specializing in particular species and areas, and focusing on greater information gathering and communication among skippers to avoid catching constraining species (Annala et al. 1991; Baelde 2001; Grafton et al. 2006). As a result of behavioral changes, the gaps between catches and TACs have decreased in multispecies fisheries in the Australian south-east trawl fishery and in New Zealand (Annala 1996; Connor and Alden 2001).

To fill the gap in quantitative research on this topic, we analyzed the behavioral changes under ITQs in the British Columbia groundfish trawl fishery in Canada (referred to throughout as the "B.C. fishery"). In this fishery, ITQs were implemented in 1997 together with 100% at-sea observer coverage and the deduction of discard mortality from quotas (Turris 2000), thus providing strong incentives to match catches to quotas while discouraging discarding. We describe the management system implemented in the B.C. fishery and examine the extent to which changes in TACs corresponded to changes in catches. Trawl data are examined at fine-scale resolution to detect whether different "fishing opportunities" (Branch et al. 2005) were avoided or targeted because of their species composition. We find that fishing opportunities containing rougheye (*Sebastes aleutianus*), shortraker (*Sebastes borealis*), and yelloweye (*Sebastes ruberrimus*) rockfish were avoided when TACs for these species were cut and that the multispecies mixture in fishing opportunities played a key role in deciding where to fish.

Fishery background

Prior to ITQs, the B.C. fishery was managed under a system of trip limits (maximum landing limits for each species or species complex). In 1995, 100% dockside monitoring of catches was introduced, but continued concerns over TAC overruns and problems with discarding led to the closure of the fishery in September 1995 (Turris 2000). When it reopened in February 1996, each vessel was required to carry an observer, except for Pacific whiting (*Merluccius productus*) boats and a small "option B" sector of the fishery (landing only 0.3%–1.7% of the total catch). Trip landing limits were replaced with nontransferable four-monthly or annual limits on total catch mortality, i.e., landings plus the estimated mortality of marketable discards. Marketability was based on size limits developed in conjunction with processors; estimated mortality of discards varied by species and trawl duration. Total mortality limits were imposed on either indi-

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vidual species or pairs of species. If a vessel exceeded the total mortality limit for any species or pair of species, that vessel would not be allowed to continue bottom trawling for the remainder of the catch period. In April 1997, after an interim January–March management period, the fishery added ITQs to this system. Shares of TACs were allocated to vessel owners based on vessel length (30%) and catch history (70%). ITQs could be bought or sold, and arrangements could be made that effectively amounted to leasing. Vessels were allowed to carry forward overruns and underruns of up to 37.5% (varying by species and year) to the following year. The 1996 requirements of full at-sea observer and dockside monitoring were retained, as was the focus on mortality limits instead of landing limits. All TACs were annual limits, and where they had been imposed on pairs of species, they were now set for individual species. TACs were imposed on 22 species (Table 1), some on an area-specific basis and some coast-wide, totaling 55 species-area TAC restrictions in 1997–1998, with only minor alterations in subsequent fishing seasons.

The B.C. fishery management plan sets a hard limit on the total catch that can be taken for each species. Thus, there are strong incentives for skippers to change their fishing behavior to match their catches to their ITQ holdings. Under this system, changes in TACs should result in targeting and avoidance behavior. If TACs increased, skippers should increase trawls in areas where those species are abundant; if TACs decreased, the areas containing the affected species should be fished less.

Materials and methods

Catch ratios

Individual trawl data were obtained from the British Columbia PacHarv database. Extracted information included start and end positions, vessel identification numbers (coded for anonymity), and catches of the top 32 species (scientific names are listed in Table 1). These species included all those under ITQ management and all non-ITQ species with average annual catches greater than 50 tonnes (t). We defined "catch" as the amount that would be subtracted from quotas, i.e., total landings plus discard mortality of marketable fish. Discard mortalities were based on the values assumed for management purposes: rockfish (*Sebastes* spp.) and thornyhead (*Sebastolobus* spp.) are assumed to suffer 100% discard mortality, but for other species, discard mortalities were lower; for example, for sablefish and the sole species, discard mortalities were assumed to be 10% for the first 2 h of trawling and 10% per hour thereafter. Annual catches (Table 1) were obtained for six fishing seasons: the pre-ITQ 1996 fishing season (12 February to 31 December) and five post-ITQ fishing seasons from 1997 to 2001 (1 April to 31 March). The interim period between the management systems (1 January to 31 March 1997) was excluded. For each species, the catch ratio, R_C, was calculated to measure the change in actual catches between 1996 and 1997–2001:

$$(1) \qquad R_C = \frac{2\overline{C}_{1997\text{–}2001}}{\overline{C}_{1997\text{–}2001} + C_{1996}} - 1$$

where C_{1996} is the catch in 1996, $\overline{C}_{1997\text{–}2001}$ is the mean annual catch in the 1997 to 2001 seasons, and $-1 \le R_C \le 1$. Positive values indicate that mean catches increased compared with 1996, whereas negative values occur if mean catches decreased after 1996.

Bounded ratios

The catch ratio is an example of a "bounded ratio" in that it lies between –1 and +1. More generally, bounded ratios could measure the change in any quantity from one time period to the next. Bounded ratios have several advantages over simple ratios (e.g., $\overline{C}_{1997\text{–}2001}/C_{1996}$): (*i*) they are defined even when one quantity is zero; (*ii*) they are symmetric with respect to the two quantities of interest, e.g., if C_{1996} and $\overline{C}_{1997\text{–}2001}$ are switched, the sign of the bounded ratio changes but the absolute value remains the same; (*iii*) the contrast between negative and positive values is more intuitive than contrasts between values in $[0, 1]$ and $[1, \infty)$; and (*iv*) they have better numerical properties than simple ratios; for example, the mean of bounded ratios is robust to high or low values, whereas the mean of simple ratios can be dominated by one high value if, for example, C_{1996} was close to zero.

For these reasons, similarly defined bounded ratios are used throughout this paper to measure TAC ratios, utilization ratios, and multispecies catch ratios.

TAC ratios

TAC ratios measure the change in TACs from 1996 to 1997–2001 (Table 2) and are analogous to the catch ratios. For each species, the TAC ratio, R_T, is defined by

$$(2) \qquad R_T = \frac{2\overline{T}_{1997\text{–}2001}}{\overline{T}_{1997\text{–}2001} + T_{1996}} - 1$$

where T_{1996} is the TAC in 1996, and $\overline{T}_{1997\text{–}2001}$ is the mean TAC in the 1997 to 2001 seasons. The TAC ratio is bounded by –1 and 1 and will be positive if the mean TAC increased after 1996, and negative otherwise.

Note that the difference between TAC ratios and catch ratios, as defined above, is that catch ratios are a measure of how much the actual observed catch changed from 1996 to 1997–2001 and the TAC ratio is a measure of the permissible change in allowed catches from 1996 to 1997–2001, i.e., the change in incentive to target or avoid particular species. Of course, if changes in TACs perfectly match changes in the actual stock sizes, then no change in targeting or avoidance would be required, but in practice, changes in TACs usually lag changes in stock size by several years because of the time required to analyze data, conduct stock assessments, and decide on new TAC values. Additionally, changes in TACs are often driven by politics or by changes in management goals, rather than being based solely on biological considerations.

For many species, TAC ratios could not be obtained directly. However, TAC ratios can be thought of as the relative incentive to target or avoid a species: positive values indicate increased incentives to target that species (relative to 1996) and negative values indicate that the species should be avoided compared with 1996. Where regulations have changed, species can be classified into those that should be preferentially targeted ($R_T > 0$) and those that should be

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Table 1. Yearly coast-wide catch and catch ratio for each species in the B.C. groundfish fishery.

		Catch (tonnes)						
Common name	Scientific name	1996	1997	1998	1999	2000	2001	Catch ratio
Arrowtooth flounder	*Atheresthes stomias*	4 293	2 573	3 720	3 626	4 391	7 381	0.00
Big skate	*Raja binoculata*	274	482	456	892	624	1 201	0.46
Bocaccio	*Sebastes paucispinis*	240	236	194	217	239	234	–0.04
Canary rockfish	*Sebastes pinniger*	539	761	755	920	800	823	0.20
Darkblotched rockfish	*Sebastes crameri*	108	71	55	82	84	77	–0.18
Dover sole	*Microstomus pacificus*	2 791	2 454	2 647	2 984	3 054	3 173	0.02
English sole = lemon sole	*Parophrys vetulus*	635	674	669	749	676	608	0.04
Lingcod	*Ophiodon elongatus*	1 764	1160	802	1 095	1 756	1257	–0.18
Longnose skate	*Raja rhina*	143	149	110	203	114	172	0.02
Longspine thornyhead	*Sebastolobus altivelis*	811	518	755	871	801	624	–0.06
Pacific cod	*Gadus macrocephalus*	598	1 249	926	669	620	544	0.14
Pacific hake = Pacific whiting	*Merluccius productus*	29 667	50 243	50 930	68 229	10 383	35 341	0.18
Pacific halibut	*Hippoglossus stenolepis*	357	276	234	214	272	205	–0.20
Pacific ocean perch	*Sebastes alutus*	6 173	5 645	5 572	6 034	5 812	5 675	–0.04
Petrale sole	*Eopsetta jordani*	280	354	339	347	423	452	0.16
Redbanded rockfish	*Sebastes babcocki*	332	237	191	288	268	271	–0.14
Redstripe rockfish	*Sebastes proriger*	924	895	866	1 097	1 104	1 025	0.04
Rex sole	*Glyptocephalus zachirus*	210	204	257	308	322	325	0.14
Rock sole	*Lepidopsetta bilineatus*	1 091	959	898	1 068	1 155	1 091	–0.02
Rougheye rockfish	*Sebastes aleutianus*	1 068	489	597	494	448	464	–0.36
Sablefish	*Anoplopoma fimbria*	227	246	333	392	330	315	0.18
Sharpchin rockfish	*Sebastes zacentrus*	140	123	112	233	325	370	0.24
Shortraker rockfish	*Sebastes borealis*	144	59	41	62	64	76	–0.40
Shortspine thornyhead	*Sebastolobus alascanus*	731	515	518	798	682	569	–0.08
Silvergray rockfish	*Sebastes brevispinis*	1 164	1 167	1 226	1 510	1 192	916	0.02
Spiny dogfish	*Squalus acanthias*	196	97	157	185	215	173	–0.08
Splitnose rockfish	*Sebastes diploproa*	44	64	51	60	71	54	0.16
Walleye pollock	*Theragra chalcogramma*	1 274	523	750	1 122	894	379	–0.26
Widow rockfish	*Sebastes entomelas*	1 712	1 595	1 653	1 846	1 891	2 215	0.04
Yelloweye rockfish	*Sebastes ruberrimus*	48	13	15	17	24	15	–0.48
Yellowmouth rockfish	*Sebastes reedi*	1 649	2 206	2 090	2 368	2 120	2 209	0.14
Yellowtail rockfish	*Sebastes flavidus*	5 181	3 409	3 797	4 807	3 670	3 638	–0.14

preferentially avoided ($R_I < 0$). Following this logic, TAC ratios were calculated or assigned as follows for each group of species (Table 2). (1) Individual species TACs were available in all years (four species): TAC ratio was calculated directly. (2) TACs were available for pairs of rockfish species in 1996 and for individual species in 1997 to 2001 (eight species). The 1996 TAC was divided between the two species based on the 1996 catch proportions of those species before the TAC ratios were calculated. (3) TAC was available for a pair of species in 1996, but under trip limits for all minor rockfish species thereafter (sharpchin rockfish). Because the trip limit was less restrictive than the TAC, there was an increased incentive to target this species; hence, $R_I > 0$. (4) TAC in 1996 was applied to a limited region, whereas TAC in 1997 to 2001 was coastwide (three species). For these species, total coastwide catches in 1996 were smaller than the TACs in 1997 to 2001; therefore $R_I > 0$. (5) Species could be landed only as bycatch in 1996, but under TACs for 1997 to 2001 (two species). Because restrictions were reduced, $R_I > 0$. (6) There was no TAC in any season (four species), the development of markets for these species would be expected, hence increasing targeting; therefore $R_I > 0$. (7) For minor rockfish (four species), there was no restriction in 1996 but a combined species trip limit of 5000 pounds (2.27 t) in 1997 and 15 000 pounds (6.80 t) in 1998–2001. Increased avoidance would be expected; hence $R_I < 0$. (8) Catches were less than 20% of TACs (spiny dogfish and walleye pollock). These species are low value, difficult to process, and would generally be avoided; hence, $R_I < 0$. (9) There was no TAC in 1996 and TAC only imposed in 1997 to 2001 (longspine thornyhead). Avoidance would be expected; hence, $R_I < 0$. (10) Pacific halibut was required to be discarded, but each vessel was assigned tradeable bycatch limits that were reduced after 1996; yelloweye rockfish was required to be relinquished from 1998 onwards with a target of, at most, 16 t after catches of 48 t in 1996. Increasing avoidance of these species would be expected; hence, $R_I < 0$.

Relationship between TAC ratios and catch ratios

A positive TAC ratio reflects an increasing incentive to catch that species between 1996 and 1997–2001; a negative TAC ratio indicates greater incentive to avoid that species. Catch ratios reflect the actual change in catches: positive values indicate that catches increased, negative values indicate that catches decreased. The Spearman rank correlation

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Table 2. Coast-wide total allowable catches (TAC) and TAC ratios for species in the B.C. groundfish fishery in 1996–2001.

	TAC (tonnes)						
Species	1996	1997	1998	1999	2000	2001	TAC ratio
Arrowtooth flounder	None	None	None	None	None	None	Positive
Big skate	None	None	None	None	None	None	Positive
Bocaccio	None	Trip	Trip	Trip	Trip	Trip	Negative
Canary rockfish (a)	660	929	929	921	1 097	1 046	0.20
Darkblotched rockfish	None	Trip	Trip	Trip	Trip	Trip	Negative
Dover sole*	2 913	3 073	3 073	3 073	3 073	3 073	Positive
English sole*	493	791	791	771	771	730	Positive
Lingcod	5 115	2 887	2 500	2 462	2 462	2 462	–0.34
Longnose skate	None	None	None	None	None	None	Positive
Longspine thornyhead	None	860	861	855	404	405	Negative
Pacific cod	Bycatch	2 574	1 954	1 954	1 954	1 154	Positive
Pacific hake	53 000	114 600	94 687	90 300	90 300	81 600	0.28
Pacific halibut	Reling.	Reling.	Reling.	Reling.	Reling.	Reling.	Negative
Pacific ocean perch (b)	5 433	6 481	6 147	6 147	6 147	6 147	0.06
Petrale sole	Bycatch	479	479	479	479	479	Positive
Redbanded rockfish	None	Trip	Trip	Trip	Trip	Trip	Negative
Redstripe rockfish (c)	1 757	1 623	1 564	1 562	1 562	1 521	–0.06
Rex sole	None	None	None	None	None	None	Positive
Rock sole*	1 553	2 082	2 082	2 022	2 022	1 650	Positive
Rougheye rockfish (d)	1 156	380	549	433	431	530	–0.42
Sablefish	304	386	386	386	339	339	0.10
Sharpchin rockfish (c)	267	Trip	Trip	Trip	Trip	Trip	Positive
Shortraker rockfish (d)	155	77	117	92	94	105	–0.24
Shortspine thornyhead	752	748	749	732	733	736	0.00
Silvergray rockfish (a)	1 425	1 510	1 510	1 498	1 373	1 240	0.00
Spiny dogfish†	17 000	5 440	5 440	5 440	5 440	5 440	Negative
Splitnose rockfish	None	Trip	Trip	Trip	Trip	Trip	Negative
Walleye pollock†	6 578	4 000	3 730	4 225	4 225	4 225	Negative
Widow rockfish (e)	1 921	2 358	2 157	2 157	2 358	2 316	0.08
Yelloweye rockfish	None	16	Reling.	Reling.	Reling.	Reling.	Negative
Yellowmouth rockfish (b)	1 451	2 430	2 385	2 407	2 408	2 365	0.24
Yellowtail rockfish (e)	5 813	5 233	4 464	4 464	4 464	4 422	–0.12

Note: TAC ratios could not be estimated for some species, which were indicated by "positive" if regulations increased incentives to target them or "negative" if regulations decreased the incentives to target them. Reling., required to be relinquished; Trip, combined trip limit for minor rockfish species; None, no TAC limit; Bycatch, no TAC set but catches allowed as bycatch only. The 1996 TAC is combined for species followed by the same letter (a, b, c, d, e); such TACs are divided based on 1997–2001 species-specific TACs.

*TAC for 1996 only applied to part of the coast.

†Catches <20% of TAC, TAC not constraining.

(r_S; Zar 1996, pp. 389–392) between the TAC ratios and catch ratios was obtained for species in categories 1 and 2 above, where TAC ratios could be obtained directly. A correlation coefficient of 1 would indicate perfect ability to change fishing to match catches to quota, while a correlation near 0 would suggest that fishing behavior was little changed.

Where TAC ratios could not be obtained directly, categories 3 to 10 above, species were divided into two groups: those with positive TAC ratios ($n_1 = 10$), and those with negative TAC ratios ($n_2 = 9$). A randomization test was conducted to detect whether the mean catch ratios (μ_1 and μ_2) were significantly greater for species with positive TAC ratios than for those with negative TAC ratios. The randomization test consisted of repeating the following process $N = 100\,000$ times, starting with $m = 0$: from the pool of $n_1 + n_2$ catch ratios, select with replacement one set of size n_1 and one set of size n_2. Calculate the mean catch ratios for these two sets (x_1 and x_2, respectively). Increment m if $x_1 - x_2 \geq \mu_1 - \mu_2$. The probability of obtaining the observed difference (or greater) in catch ratios between the two groups, if the null hypothesis is true, is m/N.

Defining fishing opportunities

The choice of fishing location is an obvious way to alter the mix of species that are caught. Each skipper chooses whether to fish in a known "fishing opportunity" or to go exploratory fishing; typically, skippers will use the same gear and fish in the same season whenever they fish at a given fishing opportunity. Here we follow the definition of Branch et al. (2005) that a fishing opportunity is a vessel-specific group of trawls that are clustered together over a portion of the same fishing ground. In the B.C. fishery, trawls repeated over 1996–2001 generally caught predictable

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mixtures of species in each fishing opportunity (Branch et al. 2005). The method for obtaining fishing opportunities from individual trawl start and end positions is explained in detail in Branch et al. (2005). In brief, trawls are clustered together using hierarchical agglomerative clustering (Venables and Ripley 1999, pp. 336–338), and then the resulting cluster tree is divided into subclusters (termed fishing opportunities) using a cut point of 0.15 (Branch et al. 2005). The cut point is obtained from the mean trawl length of 10.8 km using the formula $\frac{360\sqrt{2}\,10.8}{40075} = 0.14$, and the result is rounded to 0.15.

Fishing opportunities (with this cut point of 0.15) were obtained separately for each of the 34 vessels that conducted at least 100 trawls per year in the fishery. All subsequent analyses in this paper used data from these vessels only. For each vessel, the resulting fishing opportunities contained a variety of numbers of trawls, but the analyses were restricted to those fishing opportunities with ≥15 trawls (from all years combined), except for plots of the number of fishing opportunities with ≥1, 5, 10, 15, and 20 trawls. The number of fishing opportunities visited by each vessel gives an indication of the variety of places that are required to be fished to maximize economic yield from the fishery.

Utilization ratio

A utilization ratio, $R_{U,i}$, was calculated to quantify whether the proportion of trawls in each fishing opportunity increased or decreased from 1996 to 1997–2001. Utilization ratios are based on the proportion of trawls in a year that were conducted in a fishing opportunity, not the total number of trawls, because the number of trawls increased each year for the top 34 vessels (Fig. 1). This change occurred as some vessels exited the fishery (reducing the number of active vessels from 108 in 1996 to 72–75 in 1997–2001), and the remaining vessels increased their quota holdings. The utilization ratio for each fishing opportunity was defined as follows:

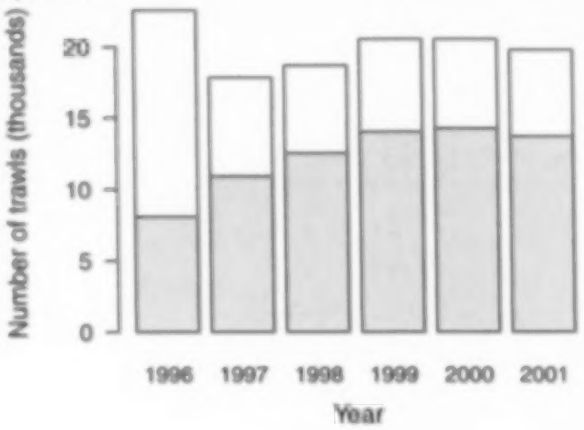
$$p_{y,i} = \frac{N_{y,i}}{\sum_i N_{y,i}}$$

$$R_{U,i} = \frac{2\bar{p}_{1997\text{–}2001,i}}{p_{1996,i} + \bar{p}_{1997\text{–}2001,i}} - 1 \qquad (3)$$

where $N_{y,i}$ is the number of trawls in year y in fishing opportunity i; $p_{y,i}$ is the proportion of trawls by a particular vessel conducted in year y in fishing opportunity i; $p_{1996,i}$ is the proportion of trawls in fishing opportunity i in 1996; and $\bar{p}_{1997\text{–}2001,i}$ is the mean proportion of trawls in fishing opportunity i in 1997 to 2001.

The utilization ratio measures whether a given vessel increased utilization (the proportion of trawls) on a particular fishing opportunity in 1997–2001 (positive R_U) or decreased utilization (negative R_U) compared with 1996. New (exploratory) fishing opportunities, where no trawls were conducted in 1996, have a utilization ratio of +1, whereas "defunct" fishing opportunities, which were not trawled in 1997–2001, have a utilization ratio of –1.

Fig. 1. Number of trawls conducted in each year in the B.C. fishery by the top 34 vessels (shaded bars) and by the remaining vessels (open bars).



Evidence for avoidance of shortraker, rougheye, and yelloweye rockfish

Avoidance of fishing opportunities containing particular species is most likely to be demonstrated when the TACs of those species were sharply decreased. In the B.C. fishery, avoidance incentives were strongest for three species: shortraker rockfish, rougheye rockfish, and yelloweye rockfish. TACs for shortraker and rougheye rockfish decreased by 60% and 38%, respectively, after 1996 (Table 2). These two species were analyzed separately even though they can be difficult to discriminate at sea (Love et al. 2002). The third species, yelloweye rockfish, had no species-specific restrictions in 1996 (when 48 t were caught), but the TAC for 1997 was set at 16 t, and thereafter they were required to be relinquished with total catch targets ranging from 0 to 14 t.

CPUE (catch per trawl) of these three species by the top 34 vessels were calculated. CPUE should be lower in 1997–2001 than in 1996 if fishing patterns changed to avoid catching these species, although technology improvements would tend to increase CPUE over time. For a more detailed examination, the proportion of trawls that caught nonzero, medium, and large quantities of these species in each year were plotted to see whether fishers actively avoided any habitat where these species occur, or only avoided areas where larger catches might be expected. Trawls above the medium threshold accounted for 75% of the total catch of that species, whereas trawls above the large threshold accounted for 25% of the total catch of that species.

To detect any avoidance of shortraker, rougheye, or yelloweye rockfish, their proportions were calculated as a percentage of the total catch at each fishing opportunity (summed over trawls and species). Utilization ratios were then calculated for categories of fishing opportunities containing different proportions of each of these species. Randomization tests were conducted for each species to see if utilization ratios were significantly lower in fishing opportunities where ≥1% of each of these three species were caught than in fishing opportunities where <1% were caught.

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Fig. 2. Relationship between the total allowable catch (TAC) ratio, R_T, and the catch ratio, R_C, for the 13 species for which the TAC ratio could be calculated. The 1:1 line is shown.

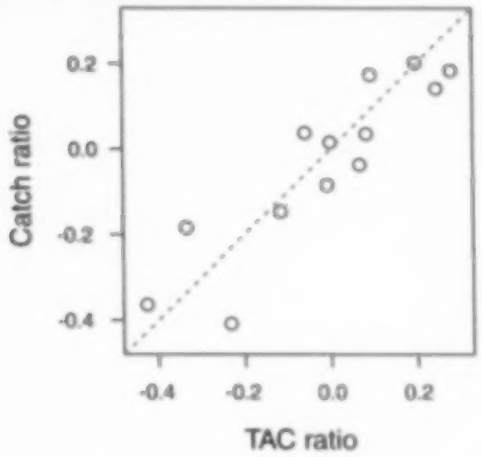
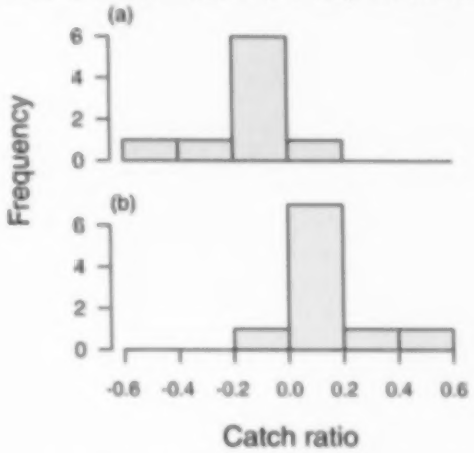


Fig. 3. Frequency distribution of catch ratios, R_C, among species for which total allowable catch (TAC) ratios, R_T, could not be calculated but which are presumed to fall into two groups: (*a*) those with positive TAC ratios and (*b*) those with negative TAC ratios.



Multispecies TAC ratios

A multispecies TAC ratio was developed based on the 13 species for which TAC ratios could be calculated directly. For each fishing opportunity, we combined the proportions (p_i) of each species i with the species-specific TAC ratios ($R_{T,i}$) to obtain multispecies TAC ratios, R_M, for each fishing opportunity:

$$p_i = \frac{\sum_t C_{i,t}}{\sum_i \sum_t C_{i,t}} \qquad R_M = \sum_i p_i R_{T,i} \tag{4}$$

where $C_{i,t}$ is the catch of species i in trawl t at a fishing opportunity.

A randomization test was conducted to determine whether the mean utilization ratio differed significantly for fishing opportunities with positive and negative multispecies TAC ratios. Additionally, new fishing opportunities (those not fished in 1996) were analyzed to determine whether a greater proportion than expected had positive multispecies TAC ratios, using the Fisher exact test (Zar 1996, pp. 540–547).

Results

TAC ratios and catch ratios

For those species for which TAC ratios could be calculated directly, TAC ratios and catch ratios were highly correlated ($r_S = 0.90$, $n = 13$, $P < 0.001$), indicating that when TACs were increased, catches of the corresponding species increased proportionately, and when TACs decreased, catches decreased accordingly (Fig. 2).

A similar trend was apparent for those species for which the TAC ratio could not be calculated directly (Fig. 3). Nine out of 10 species with positive TAC ratios had positive catch ratios (mean 0.12, range –0.03 to 0.46); and eight out of nine species with a negative TAC ratio also had negative catch ratios (mean –0.14, range –0.49 to 0.15). The lowest catch ratio was for yelloweye rockfish (–0.49), and the highest catch ratio was for big skate (0.46). Among these 19 species, catch ratios were significantly greater for species with positive TAC ratios than for species with negative TAC ratios ($n_1 = 10$, $n_2 = 9$, $\mu_1 = 0.12$, $\mu_2 = -0.14$, $P = 0.002$, randomization test).

Fishing opportunities

Each of the top 34 vessels conducted one or more trawls at a diverse range of fishing opportunities (mean 129, range 60–250; Fig. 4), including exploratory fishing opportunities and possible positional errors in the locations (Branch et al. 2005). When the threshold was increased to 15 trawls, each vessel still fished at a wide range of fishing opportunities (mean 38, range 20–69; Fig. 4). Of the 1302 fishing opportunities with 15 or more trawls, 585 (45%) were new fishing opportunities (not fished in 1996) and 11 (0.8%) were defunct fishing opportunities (not fished in 1997–2001).

Avoidance of shortraker, rougheye, and yelloweye rockfish

From 1996 to 1997–2001, CPUE of rougheye, shortraker, and yelloweye rockfish decreased by 56%, 59%, and 65%, respectively (Fig. 5), mirroring the TAC cuts for these species. The percentage of trawls that caught nonzero quantities of these species was lower in 1997–2001 for rougheye and shortraker rockfish, but not for yelloweye rockfish, and there were large decreases in the percentages of trawls that caught medium and large quantities of all three species in 1997–2001 (Fig. 6).

There was good evidence for the avoidance of fishing opportunities where rougheye, shortraker, and yelloweye rockfish were likely to be caught. Fishing opportunities where these species comprised ≥1% of the total catch had significantly lower utilization ratios (Fig. 7) for rougheye rockfish ($n_1 = 1089$, $n_2 = 213$, $\mu_1 = 0.47$, $\mu_2 = 0.19$, $P < 0.0001$) and shortraker rockfish ($n_1 = 1207$, $n_2 = 95$, $\mu_1 = 0.44$, $\mu_2 =$

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Fig. 4. The frequency distribution of the number of fishing opportunities visited by each of the top 34 vessels in 1996–2001, with (*a*) ≥1, (*b*) ≥5, (*c*) ≥10, (*d*) ≥15, and (*e*) ≥20 trawls in each fishing opportunity.

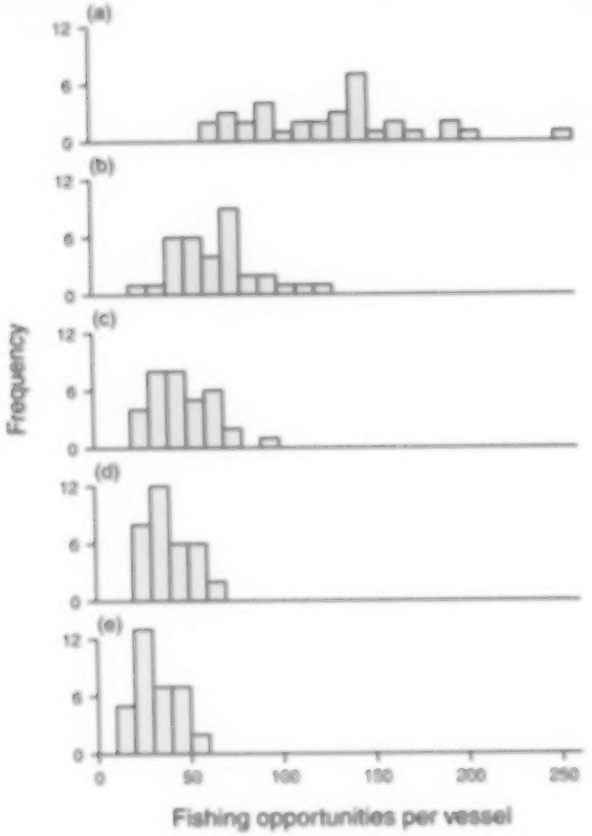
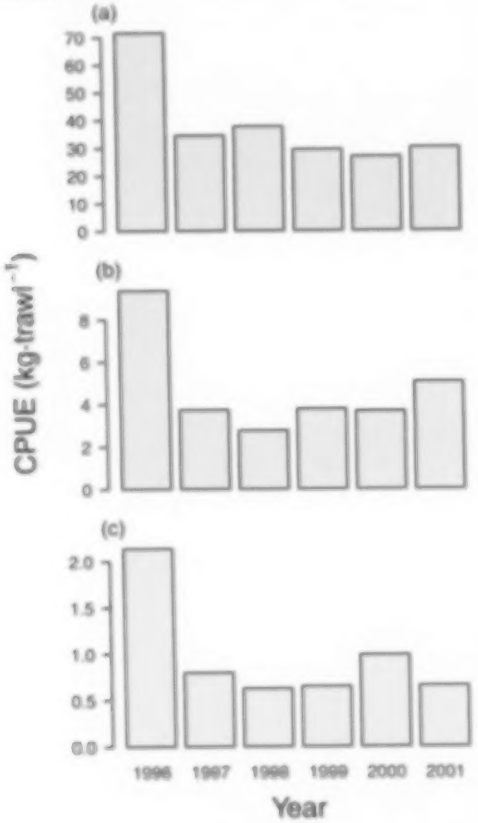


Fig. 5. Catch per unit effort (CPUE, kg per trawl) of (*a*) rougheye (*Sebastes aleutianus*), (*b*) shortraker (*S. borealis*), and (*c*) yelloweye (*S. ruberrimus*) rockfish for the top 34 vessels in the B.C. fishery. Allowable catches were substantially reduced for all three species in 1997–2001.



0.15, $P < 0.0001$), but not for yelloweye rockfish ($n_1 = 1292$, $n_2 = 10$, $\mu_1 = 0.43$, $\mu_2 = 0.13$, $P = 0.08$). However, the nonsignificant result for yelloweye rockfish is probably due to the small number (10) of fishing opportunities in which more than 1% of the catches were yelloweye rockfish.

Multispecies TAC ratios and utilization ratios among fishing opportunities

The mean utilization ratio was significantly higher when multispecies TAC ratios were positive than when they were negative ($n_1 = 603$, $n_2 = 698$, $\mu_1 = 0.50$, $\mu_2 = 0.36$, $P < 0.0001$, randomization test). New fishing opportunities were significantly more likely than expected to have positive multispecies TAC ratios (Fisher exact test, $P < 0.0001$).

Discussion

If fishers can control the species composition in their catches, then multispecies fisheries can be managed to optimize economic benefits without overfishing any species (Ricker 1958; Paulik et al. 1967; Hilborn et al. 2004). Usually skippers do have some ability to control their catch mixture, thereby increasing yields with lower than predicted risks of overfishing, but most management systems provide neither the incentives nor the flexibility for fishing fleets to match catches of individual species to the corresponding TACs. For example, open-access and competitive fisheries encourage overcapitalization and a race for fish, leading to a shortened season and reduced fishing flexibility. Input controls, effort restrictions, and area closures act by reducing fishing effort but also limit flexibility. In contrast, ITQ systems increase fishing flexibility by reducing restrictions on fishing gear, allowing year-round fishing, and enabling the leasing of quota to cover overages, while quota constraints provide incentives to keep catches within TACs. Nevertheless, in many multispecies ITQ systems, there have been problems in matching catches to quota, resulting in incentives to underreport or discard catches that are over quota (Copes 1986; Annala et al. 1991; Squires et al. 1998), although these problems generally decrease over time as fishing patterns change (Annala 1996; Connor and Alden 2001).

The B.C. fishery is managed under an ITQ system that provides incentives for skippers to match their catches to in-

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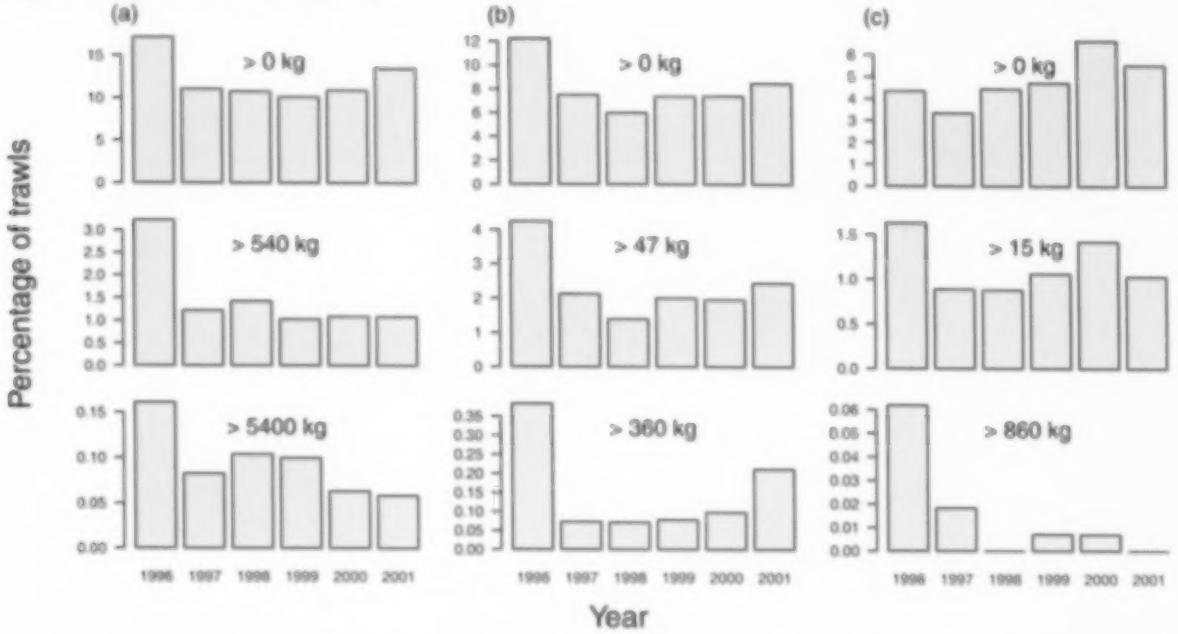
Fig. 6. The proportion of trawls by the top 34 vessels in 1996–2001 that caught small, medium, and large quantities of (*a*) rougheye (*Sebastes aleutianus*), (*b*) shortraker (*S. borealis*), and (*c*) yelloweye (*S. ruberrimus*) rockfish. The chosen thresholds for "medium" and "large" quantities cumulatively summed to 75% and 25%, respectively, of the total catches of each species. Note that the scales on the y axes are different for each individual graph.



dividual-species quotas and also minimizes misreporting, high-grading, and discarding by placing observers on board each vessel and deducting discard mortality of marketable fish from quotas (Turris 2000; Branch et al. 2006*b*; Grafton et al. 2007). Unlike some multispecies ITQs, discards in this fishery have decreased over time and are lower than in the adjacent US west coast groundfish trawl fishery, which is managed by trip limits (Branch 2006; Branch et al. 2006*b*). In the B.C. fishery, profits are maximized by fully catching quotas for as many species as possible (or leasing the quota to others) while minimizing fishing costs. To the extent that it is possible, fishers should therefore avoid catching species with constraining TACs while targeting (valuable) species with nonconstraining TACs.

Fishers in the B.C. fishery have shown a remarkable ability to adjust the mixture of species in their catches to match changes in TACs from 1996 to the later years (1997–2001). The changes in TACs (TAC ratios) were highly correlated (r_S = 0.90) with corresponding changes in catches (measured by catch ratios) for species for which the TAC ratio could be measured directly. For species for which the TAC ratio could not be measured directly, but "positive" or "negative" incentives to change catches were inferred, catch ratios also showed evidence of targeting and avoidance in a manner that was unlikely to be due to chance alone (P = 0.002). These results provide evidence for changes in fishing behavior to maximize catches of all species. A narrowing of the gap between catches and TACs has also been reported in the Australian south-east trawl fishery under ITQs as fishers adjusted their fishing methods to better catch the TACs (Connor and Alden 2001); however, that fishery has been plagued by discarding (Baelde 2001; Punt et al. 2006), so that their success may not fully reflect total fishing mortality as it does in the B.C. fishery.

There are a variety of methods that skippers can use to adjust the species proportions in their catches, including fishing location, depth, gear type, time of day, month, trawl duration, and mesh size. Our analysis shows that location choice (choice of fishing opportunities) can partially explain how fishers adjust their catch composition, although more detailed analyses including these other factors would further improve our understanding. We examined changes in the proportion of trawls at fishing opportunities (collections of trawls by a single vessel over the same portion of fishing ground; Branch et al. 2005). Trawls are commonly grouped in this manner, as can be seen in the US west coast groundfish trawl fishery (Bellman et al. 2005) and in the Dutch beam trawl fleet (Rijnsdorp et al. 2000). Trawls within fishing opportunities are usually conducted at the same time of the year, with similar gear and trawl duration, and catch similar species mixtures (Branch et al. 2005). In the B.C. fishery, the top 34 vessels each conducted ≥15 trawls in a wide variety of fishing opportunities (mean 38, range 20–69) over the period of interest (1996–2001). Many previous studies on location choice have been restricted to a few (<15) large fishing areas (e.g., Hilborn and Ledbetter 1979; Eales and Wilen 1986; Gillis et al. 1993), and these areas are far larger than the actual scale at which fishers choose to deploy their gear. A few recent studies have, however, examined fishing locations at smaller scales, for example, Pelletier and Ferraris (2000) described 28 fishing locations in a Senegalese artisanal fishery, and Durrenberger and Páls-

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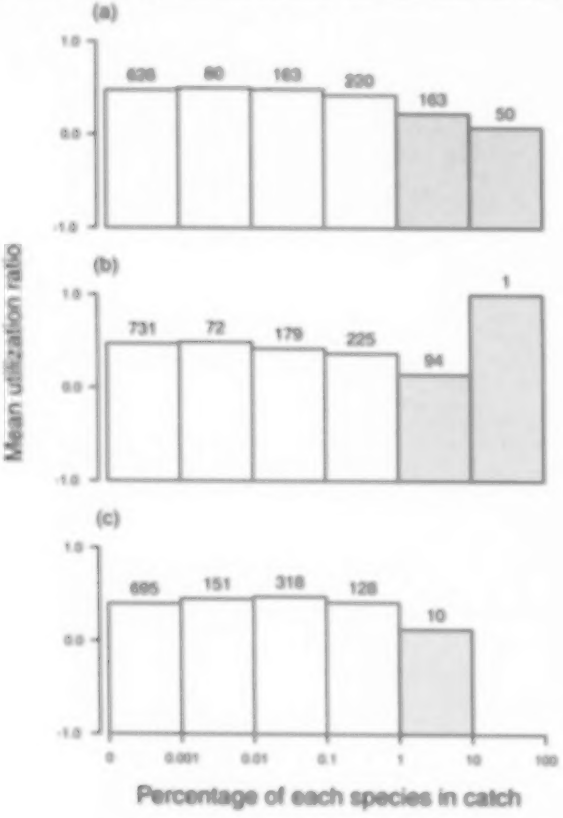

Fig. 7. The mean utilization ratio, R_U, in fishing opportunities with different percentages of (*a*) rougheye (*Sebastes aleutianus*), (*b*) shortraker (*S. borealis*), and (*c*) yelloweye (*S. ruberrimus*) rockfish. Open bars indicate fishing opportunities where the catch of these species was <1%; shaded bars are where those species were ≥1% of the catches. For example, in 626 fishing opportunities, the catch of rougheye rockfish was <0.001% of the total catch, and for those fishing opportunities, the mean utilization ratio was 0.48.

son (1986) looked at 15 × 15 km fishing grounds used for Icelandic cod, finding that 39% of fishers fished in only one location, although a few fished in many locations. These studies found fewer fishing areas than in the B.C. fishery but were based on larger areas than the fishing opportunities here. In contrast, Rijnsdorp et al. (1998) analyzed continuous fishing positions in 5.6 × 5.6 km grids for the Dutch trawl fleet, showing that 25 sampled vessels fished in a mean of 957 locations (range 92–2120), many more than the fishing opportunities in the B.C. fishery. However, in Rijnsdorp et al.'s study, a single trawl could be recorded in multiple grid cells, and exploratory trawls would additionally be counted towards the total, whereas the method used here requires repeated (15) trawls in each fishing opportunity. The relatively high number of fishing opportunities per vessel in the B.C. fishery is likely related to the large number (55) of species–area TAC combinations, requiring fishers to spread their catches over a variety of different areas to maximize their catches of all species.

In the B.C. fishery, 45% of the fishing opportunities were classified as new (not fished in 1996), and only 0.8% were classified as defunct (not fished after 1996). The high proportion of new fishing opportunities may be due to an expansion of fishing into deeper waters on the Dover sole, thornyhead, and sablefish complex, but it may also be due to ITQ implementation in 1997 encouraging fishers to find new fishing opportunities where constraining species were less likely to be caught. The relatively high number of new fishing opportunities may also reflect the threshold of 15 trawls for defining a fishing opportunity; the top 34 vessels conducted fewer trawls in 1996, and it would have been easier to accumulate 15 trawls over the 5-year period 1997–2001 than in the single year of 1996.

The B.C. fishery provides clear evidence of the avoidance of rougheye, shortraker, and yelloweye rockfish when TACs for these species were substantially reduced. Fishers decreased CPUE of these species by more than 50% from 1996 to 1997–2001, mainly by reducing the percentages of trawls that caught medium and large quantities of these species. It could be argued that the decline in CPUE was due to a decline in the abundance of the species and not due to any change in fleet behavior. However, this could only be true if the managers were able to predict a dramatic decrease in abundance in the exact year in which they implemented a TAC reduction, which seems unlikely. It is more plausible that a change in fishing behavior caused the decrease in CPUE. Evidence presented here shows that vessels tended to avoid fishing opportunities where rougheye, shortraker, and yelloweye rockfish were more abundant: mean utilization ratios were substantially lower (0.13–0.19 vs. 0.43–0.49) in fishing opportunities where more than 1% of the total catch comprised one of these species. These utilization ratios were significantly lower ($P < 0.0001$) for rougheye and shortraker rockfish, but not quite significant ($P = 0.08$) for yelloweye rockfish, probably because there were few fishing opportunities where yelloweye rockfish catches exceeded 1% of the total.

Extending the utilization analysis to the 13 species for which TAC ratios could be calculated found similar results, with mean utilization ratios being significantly lower (0.36 vs. 0.50) for fishing opportunities with negative multispecies TAC ratios. Part of the explanation is that more new fishing opportunities than expected by chance had positive multispecies TAC ratios; in other words, fishers actively developed new fishing grounds containing species for which TACs had increased.

It should be said, of course, that changes in TACs are not the only factors affecting where and how fishers choose to fish. Other important factors include the expected size of the catch at a fishing opportunity, quota remaining for each species, current market prices, distance from the port, local knowledge, and weather conditions.

In conclusion, the ITQ system in the B.C. fishery provides incentives for the fleet to adjust their total catches (including discards) when the TACs for individual species are altered. One of the ways in which they do this is to alter the proportion of trawls they allocate to different fishing opportunities known to each fisher. Fishers used this method to reduce catches of rougheye, shortraker, and yelloweye rockfish by more than 50% when more restrictive TACs were imposed on those species, while catches for other species were in-

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creased at the same time. More generally, fishers made choices about fishing opportunities based on the multispecies mixtures in those locations, avoiding areas containing species with decreased TACs while targeting fishing opportunities containing species with increased TACs. This is just one aspect of fishing behavior, as a variety of other factors also play an important role in determining the final mixture of species landed.

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Transport and growth of larval cisco (*Coregonus artedi*) in the Keweenaw Current region of Lake Superior

Jason K. Oyadomari and Nancy A. Auer

Abstract: Larval cisco (*Coregonus artedi*) were sampled from Lake Superior off the western coast of the Keweenaw Peninsula (Michigan, USA) to determine if the Keweenaw Current influences their distribution and growth. Bongo net tows were conducted during April–June 2000 from four transects extending from 0.1 to 17 km offshore. For most sampling dates, cisco tended to be slightly more abundant, larger, and older at inshore locations. Later-hatched larvae appeared at the western-most transects first, during which time earlier-hatched larvae disappeared from the study area. This pattern followed what would be expected if larvae were being transported eastward by the prevailing Keweenaw Current. Estimated growth rates were 84% higher for later-hatched compared with earlier-hatched larvae and 25% lower for offshore larvae from the eastern-most transects compared with elsewhere. Accordingly, surface water temperatures partly explained spatial and temporal variations in growth rate. These results indicate that cisco larvae in Lake Superior may be transported great distances from major spawning sites by longshore currents and that the temperature regime of nursery areas may largely control their growth.

Résumé : Nous avons échantillonné des larves de ciscos de lac (*Coregonus artedi*) du lac Supérieur au large de la côte occidentale de la péninsule de Keweenaw (Michigan, É.-U.) afin de déterminer si le courant de Keweenaw affecte leur répartition et leur croissance. Nous avons effectué des traits de filets bongo en avril–juin 2000 sur quatre transects allant de 0,1 à 17 km de la rive. Pour la plupart des dates d'échantillonnage, les ciscos avaient tendance à être légèrement plus abondants, plus grands et plus vieux dans les sites près de la rive. Les larves à éclosion plus tardive apparaissent d'abord dans le transect situé le plus à l'ouest, au moment où les larves à éclosion plus hâtive ont disparu de la zone d'étude. Ce patron s'accorde avec ce qu'on peut attendre si les larves sont transportées vers l'est par le courant prédominant de Keweenaw. Les taux de croissance estimés chez les larves à éclosion tardive sont 84 % plus élevés que ceux des larves à éclosion hâtive et ils sont de 25 % inférieurs chez les larves du large dans le transect le plus à l'est par comparaison à tous les autres sites. En conséquence, les températures des eaux superficielles expliquent en partie les variations spatiales et temporelles des taux de croissance. Nos résultats indiquent que les larves de ciscos du lac Supérieur peuvent être transportées sur de longues distances à partir des principaux sites de fraye par les courants côtiers et que les régimes thermiques des zones de nourricerie peuvent en grande mesure contrôler leur croissance.

[Traduit par la Rédaction]

Introduction

Cisco (*Coregonus artedi*) in Lake Superior declined precipitously in the 1960s, resulting largely from sequential overfishing of discrete stocks (Lawrie and Rahrer 1973; Selgeby 1982). Decades of harvest regulation have allowed cisco stocks to rebound, but recovery has been slow and largely inhibited by highly variable recruitment (Bronte et al. 2003; Hoff 2004).

Cisco recruitment appears to be controlled by a combination of biotic and abiotic factors. Within US waters of Lake Superior, Kinnunen (1997) found that the spatial abundance of yearlings was positively correlated with surface water temperatures during their first 2 months of life. However, annual fluctuations were greater than spatial variations (Kinnunen 1997). Accordingly, recruitment success tends to be synchronized among the different stocks across the lake, independent of stock size (Bronte et al. 2003). In Wisconsin waters of Lake Superior, Hoff (2004) concluded that recruitment was suppressed by slimy sculpin (*Cottus cognatus*) predation on eggs, lake trout (*Salvelinus namaycush*) predation on young of the year, and adult cisco predation on their young, which interestingly was alleviated by higher wind speeds during the month of hatching.

Hoff's (2004) results imply that wind-driven currents may be important for reducing predation by dispersing larvae away from major spawning sites. Dispersal, especially by currents, would force larvae to encounter varying environmental conditions that could either favorably or adversely influence growth and survival.

Marine studies have long addressed the importance of hydrodynamic processes on recruitment (Norcross and Shaw 1984). Many marine fishes have been found to rely on cur-

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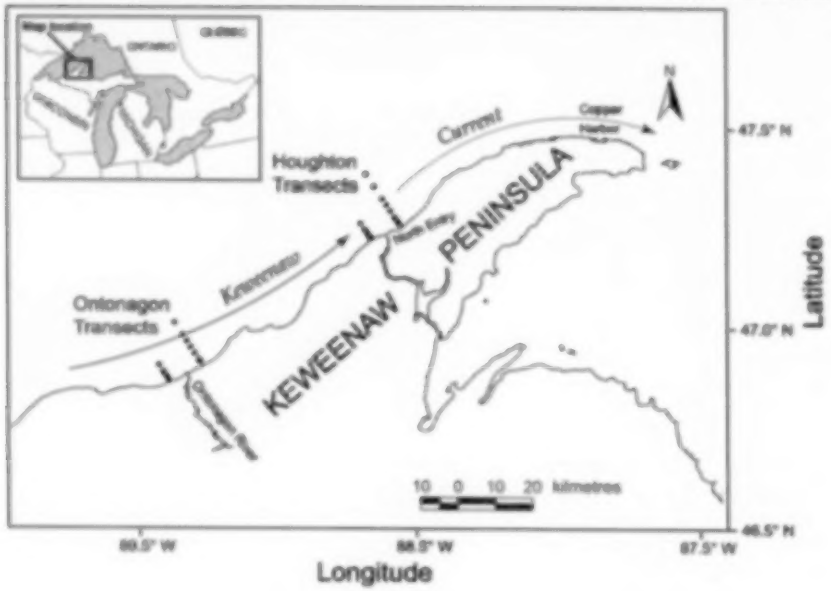
Fig. 1. Study area in Lake Superior showing the four sampling transects off the western coast of the Keweenaw Peninsula, Michigan, USA.



rents, upwellings, or riverine discharges to transport eggs or larvae from spawning grounds to nursery habitats (Norcross and Shaw 1984; Werner 2002). Consequently, anomalies in the regularity of large-scale hydrodynamic patterns may cause periodic recruitment failures (Cowan and Shaw 2002).

Freshwater lakes usually lack strong and predictable currents (Werner 2002; Moyle and Cech 2004), but the Great Lakes are large enough to exhibit oceanographic processes (Ullman et al. 1998; Beletsky et al. 1999). In particular, very large lakes tend to have regular currents in the form of a gyre (Hutchinson 1957). In Lake Superior, circulation is generally counterclockwise, with the Keweenaw Current being a particularly strong current traveling northeasterly along the western coast of the Keweenaw Peninsula, Michigan (Lam 1978; Beletsky et al. 1999). Velocities of this current can exceed 75 cm·s^{-1} in July (Niebauer et al. 1977). Despite the presence of strong and regular currents in Lake Superior, the importance of current-driven transport on cisco recruitment is unknown. The objective of this study was to determine how transport within the Keweenaw Current region of Lake Superior affects larval cisco distribution and growth, as it may relate to recruitment dynamics.

Materials and methods

Field sampling

Larval cisco were sampled from Lake Superior off the western coast of the Keweenaw Peninsula, Michigan, USA, during six cruises from April to June 2000. Net tows were conducted from four transects (Ontonagon North and South, and Houghton North and South) that ran perpendicular to the coast (Fig. 1). Dual, 70 cm diameter, 500 μm mesh bongo nets were employed to collect two samples per tow. A flow meter recorded the volume of water filtered per net. From the transects, four–nine tows were conducted from 0.1 to 17 km from shore. Locations and numbers of tows conducted varied among transects and cruises because of weather and time constraints. At each distance from shore, one tow filtered surface (top 1 m) water at a speed of 0.77–1.03 m·s^{-1} (1.5–2.0 knots) for 15 min, thus filtering on average (± standard deviation, SD) 217.3 (±76.3) m^3 of water per net. The nets were towed parallel with shore (expect for the first cruise when they were towed perpendicularly) during daylight (except for 21 May at Houghton North).

A total of 158 samples were collected with 79 tows: 28 tows from the Ontonagon transects and 51 tows from the Houghton transects. Samples were preserved with 95% ethanol so otoliths would be preserved for age analysis. A Sea-Bird Electronics conductivity–temperature–depth (CTD) meter (SBE-25) or a Hydrolab MiniSonde was used to measure vertical temperature profiles at the start of each tow and sometimes from additional distances from shore.

Laboratory measurements and otolith analysis

To determine spatial patterns in growth and development, several morphological characteristics of larval cisco were measured. In the laboratory, cisco were first separated from the samples and stored in 70% ethanol. Total length of all larvae was measured to the nearest 0.05 mm with a dial caliper. For a subsample of five randomly selected larvae per sample, or the entire sample when five or less larvae were captured, caudal fin rays were counted, and yolk depth and length were measured to the nearest 0.01 mm at 0.9× –2.0× magnification using a dissecting microscope equipped with an ocular micrometer. As an indicator of energy reserves, yolk measurements were used to calculate yolk sac volume, assuming a prolate spheroid with length as the longer axis and depth and girth as the shorter and equal axes (Luczynski et al. 1986). The number of caudal fin rays was used as an indicator of developmental stage, knowing that larvae hatch with no fin rays and progressively develop up to 28 caudal fin rays when juveniles (Hinrichs and Brooke 1975). Total

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length (TL) and yolk volume (YV) were corrected for preservation-induced shrinkage (for preservation in 70% ethanol) with linear regressions developed from laboratory-reared cisco: live TL = 1.20 + 0.91 × preserved TL and live YV = 0.50 + 0.96 × preserved YV (Oyadomari 2005).

Ages, in days, of the five randomly selected cisco per sample were estimated from otoliths following the procedures of a joint otolith validation study (Oyadomari and Auer 2007). Left and right sagittae were removed with needles under a dissecting microscope equipped with a polarizing filter. Dissected otoliths were then transferred to a microscope slide and mounted concave side down with CrystalBond (SPI Supplies), a thermoplastic mounting medium. The number of otolith increments after check formation was counted under a compound microscope using up to 1000× magnification, and counts from left and right sagittae were averaged for each larva. Age in days was then calculated from the number of otolith increments (i.e., rings) with a linear regression developed from laboratory-reared cisco: age = 28.09 + 1.04 × rings (Oyadomari and Auer 2007). Age was further used to calculate hatch dates by subtracting age from date of capture.

In order to estimate the growth rate for the last 5 days before capture using the biological intercept procedure (Campana 1990; Campana and Jones 1992), a linear relationship between fish length and otolith size had to be established, and fish length and otolith size at the biological intercept had to be determined. Otolith radius was measured as a metric of otolith size. Because cisco otoliths form from multiple primordia (Oyadomari and Auer 2007), and because having multiple primordia increases the variability in otolith nuclear dimensions (Neilson et al. 1985), the radius was measured from the posterior edge to the check mark; thus, the nuclear region was excluded. Otoliths were measured from digitally captured images with a computer image-analysis program (SigmaScan Pro 5; SPSS Inc., Chicago, Illinois), and measurements from left and right sagittae were averaged. The TL to otolith radius (RA) relationship was curvilinear, so otolith radius was square-root transformed (sqrt RA) to produce a linear relationship: TL = 8.8 + 1.31 × sqrt RA (n = 111, r^2 = 0.80, $P < 0.0001$). In this case, the biological intercept represented the point of check formation, during which the otolith radius would be zero. When there was a 50% likelihood that a larva contained a check mark, larval TL was estimated to be 11.04 mm (with 95% Fieller bounds of [10.64, 11.32]) with probit analysis (n = 232, $P < 0.0001$) (Tabachnick and Fidell 1996). Fish length at previous age a could now be estimated with the following equation: $L_a = L_c + (O_a - O_c)(L_c - L_o)(O_c - O_o)^{-1}$, where L_a is fish length at previous age a, O_a is otolith size at previous age a, L_c is fish length at capture, O_c is otolith size at capture, L_o is fish length at the biological intercept, and O_o is otolith size at the biological intercept (Campana 1990). Using this equation, larval length at 5 days before capture was predicted with square-root transformed radial length that excluded the last five increments. Finally, increase in TL during the last 5 days was expressed as a daily growth rate. For comparison, the average lifetime growth rate was also calculated for groups of larvae with length-to-age linear regressions, wherein the slope represented the daily growth rate. These two measures of growth not only differ in temporal coverage (lifetime vs. last 5 days), but also in the pool of larvae that could be employed. For recent growth, larvae had to have at least five increments, while for length-to-age growth, larvae only had to have a check mark.

Data analysis

Of the six sampling cruises during 2000, four (15–17 May, 21 May, 30 May, and 19–20 June) captured sufficient numbers of larval cisco for statistical analyses. Preliminary analyses did not show statistical differences between the north and south transects of either Ontonagon or Houghton. Accordingly, data from the two transects were pooled into a region for Ontonagon and a region for Houghton for all subsequent analyses. For some analyses, it was necessary to pool adjacent distances from shore into three transect divisions (0.1–2, 3–5, and 7–17 km from shore) to increase sample sizes. These divisions spanned different ranges so that there would be greater spatial resolution nearer to shore.

Offshore trends in density were modeled separately for each region and cruise with a posteriori curve fitting. Spatial differences in larval fish characteristics were assessed separately for each cruise with multiple linear regressions for cruises with both regions sampled and simple linear regressions for cruises with only one region sampled.

For 26% of the larvae for which otoliths were examined, age could not be estimated with otolith analysis because they had yet to produce a check mark. For these larvae, age was estimated with age-to-length linear regressions generated with data from the same cruise, region, and transect division. Although these estimated ages were probably not as accurate as ages estimated directly by otolith analysis, it was important to incorporate the younger larvae into calculations of mean age so that these means would not be biased toward the older larvae.

Hatch dates were not compared among distances from shore for each region and cruise, as described previously for the other metrics, because hatch dates are linearly dependent upon capture date (cruise) and age. Such an analysis would only show transposed results from that of age. Instead, hatch dates were compared among cruises, separately by region, with single-factor analysis of variance (ANOVA), wherein all distances from shore were pooled. However, because not all larvae of large samples were aged with otolith analysis, larger samples would be under-represented in pooled means if subsample means were weighted by their subsample sizes. Accordingly, subsample sizes were adjusted so that they were the same proportions of their total subsample size as capture numbers (from which subsamples were drawn) were to their total sample size while still maintaining the same total subsample size. In this manner, pooled means should reflect pooled populations and will not be biased by subsample sizes being unequally proportional to capture numbers. Hatch dates were also grouped into three hatch periods (early-hatch: 22 March – 8 April; bulk-hatch: 9 April – 26 April; and late-hatch: 27 April – 17 May) for some analyses. These categories included an estimated 9%, 81%, and 10%, respectively, of all captured larvae.

For each cruise, the movement or transport of larvae was assessed by graphically displaying the percentages of larvae from each hatch period for each region, transect division,

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and sampling cruise. This allowed for the assessment of where recently hatched larvae first appeared and where earlier-hatched larvae last appeared, as well as the spatial and temporal progression of recently hatched larvae replacing earlier-hatched larvae.

Growth rates estimated by length-to-age relationships were analyzed for spatial and hatch date differences with multiple regressions (Neter et al. 1996). Spatial differences were assessed by determining if the length-to-age slope varied with region and distance from shore, while hatch date differences were assessed by determining if slope varied with hatch period. Spatial differences were also assessed for back-calculated recent growth by determining if the growth rate varied with region or distance from shore (Neter et al. 1996). Age needed to be included as an independent variable in this model as well, because growth rates are typically dependent on age, and mean ages can vary spatially. Larvae for which age was estimated with total length because they were too young to age with otolith analysis were excluded from the growth rate analyses.

To determine if spatial differences in growth rates were correlated with spatial differences in temperature, growth rates were regressed on water temperature for each region. Furthermore, a temperature threshold, below which temperature was proposed to inhibit growth, was estimated by dividing the data set repeatedly by different temperatures, in 0.5 °C increments from 7 to 9 °C, instead of by region, then observing at what temperature the greatest inhibitory effect existed below it. Statistical analyses were performed with SYSTAT 10 (SPSS Inc., Chicago, Illinois), except for nonlinear regressions, which were performed with SigmaPlot 8 (SPSS Inc.).

Results

Seasonal occurrence of cisco

The sampling season commenced before the bulk of the hatch and extended to the time when larvae became rare. During the first cruise (27–29 April), surface water temperatures averaged 3.1 °C (range: 2.5–4.5 °C) (Fig. 2), and only two larval cisco were captured with 20 samples (overall mean density of 2.5 larvae·(1000 m^3)$^{-1}$). The highest densities (overall mean density of 77.0 larvae·(1000 m^3)$^{-1}$) of cisco were obtained during the second half of May, when temperatures averaged 6.0 °C (range: 3.1–8.5 °C). Catches greatly diminished by June (overall mean density of 3.4 larvae·(1000 m^3)$^{-1}$), when temperatures averaged 8.2 °C (range 4.6–11.4 °C), although there were great spatial differences in abundance during this period.

Spatial distribution in temperature, density, and larval fish size

As expected, surface water temperatures decreased with distance from shore for all cruises, and the shallower waters at Ontonagon fostered higher temperatures than those at Houghton from 21 May onward (Fig. 3). Surface waters of less than 4 °C were still observed at Houghton on 21 May from 5 km outward and on 30 May from 7 km outward, but not at Ontonagon up to 13 km from shore on 21 May.

By the second cruise, 15–17 May, cisco catches were sufficient for statistical analyses. Cisco densities during this cruise decreased linearly with distance from shore at Ontonagon (linear regression: n = 8, r^2 = 0.79, P = 0.003), but at Houghton, offshore densities most closely followed a log-normal trend (lognormal regression: n = 28, r^2 = 0.45, P = 0.001) because of zero catches at 0.25 km from shore (Fig. 3). Total lengths of larvae (multiple regression: n = 187, r^2 = 0.07), however, did not differ with region (P = 0.06), distance from shore (P = 0.20), or the interaction (P = 0.90) (Fig. 3).

During the next cruise, 21 May, we sampled to a much farther offshore extent at both Ontonagon and Houghton than previously. At Ontonagon, there were greater densities inshore than offshore up to 5 km from shore, but beyond that, we encountered a high-density patch of cisco extending to at least 13 km from shore (Fig. 3). Therefore, the offshore trend in density closely followed a quadratic equation (quadratic regression: n = 24, r^2 = 0.97, P < 0.0001). At Houghton, the trend was similarly quadratic (quadratic regression: n = 16, r^2 = 0.52, P = 0.009), although densities were substantially lower, which may have resulted, in part, from these samples being collected at night. In contrast

Fig. 2. Seasonal changes in surface water temperature in 2000 at 1 km (●), 5 km (○), and 9 km (▼) offshore on the (*a*) Ontonagon North and (*b*) Houghton North transects in Lake Superior off the western coast of the Keweenaw Peninsula, Michigan.

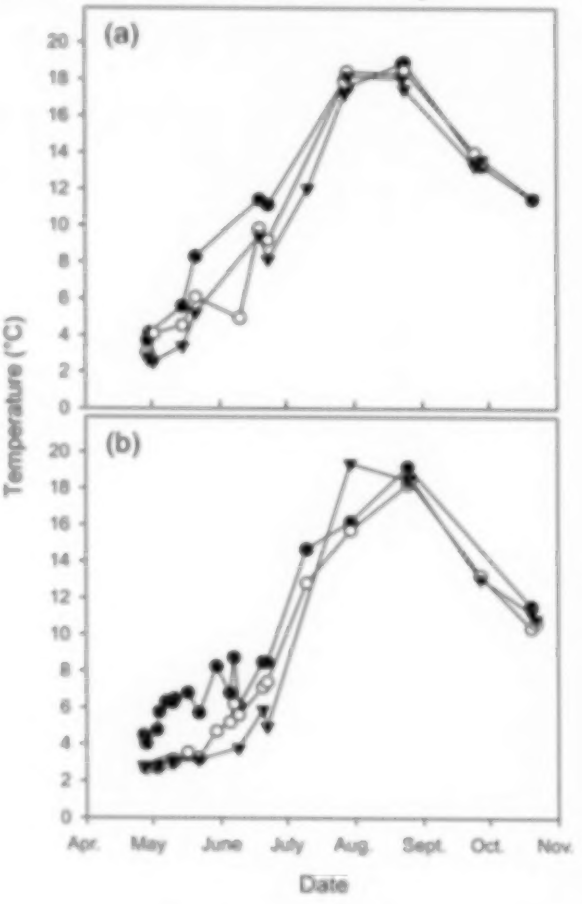

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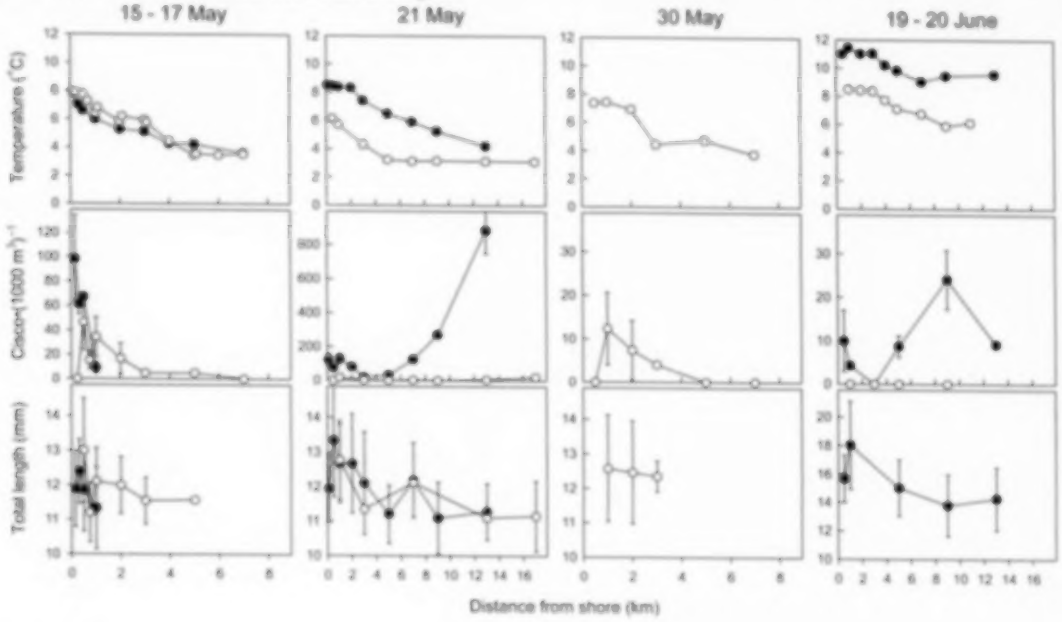
Fig. 3. Offshore trends in surface water temperature and mean (± standard deviation, SD) density and total length (TL) of larval cisco (*Coregonus artedi*) collected during four sampling cruises in 2000 from the Ontonagon (●) and Houghton (○) transects in Lake Superior off the western coast of the Keweenaw Peninsula, Michigan.



with the previous cruise, total lengths of larvae (multiple regression: $n = 1035$, $r^2 = 0.21$) now decreased with distance from shore ($P < 0.0001$), but did not differ between regions ($P = 0.66$) or for the interaction ($P = 0.81$) (Fig. 3).

On 30 May, only Houghton was sampled, and sampling extended to the same offshore extent (7 km) as for the 15–17 May cruise. By this time, densities had greatly diminished, yet interestingly, they exhibited a similar lognormal trend as previously (lognormal regression: $n = 18$, $r^2 = 0.54$, $P = 0.003$), with no larvae captured at 0.5 km from shore. Total length also did not differ with offshore distance (linear regression: $n = 21$, $r^2 = 0.003$, $P = 0.81$) (Fig. 3).

On 5–7 June, sampling was conducted only at Houghton, and only seven cisco were captured in 26 samples, so these data are not presented. On the last cruise, 19–20 June, larvae were captured only at Ontonagon. The distribution of these larvae was scattered, with peaks at 0.5–1.0 km and at 9 km from shore (Fig. 3). For total length, a decreasing trend with distance from shore was still evident (linear regression: $n = 25$, $r^2 = 16$, $P = 0.04$) (Fig. 3).

Spatial distribution in age, yolk volume, and number of caudal fin rays

Estimated ages decreased with distance from shore on 21 May (multiple regression: $n = 116$, $r^2 = 0.24$, $P < 0.0001$ for distance from shore, $P = 0.71$ for region, $P = 0.22$ for the interaction) and 19–20 June (linear regression: $n = 22$, $r^2 = 0.20$, $P = 0.04$) (Fig. 4). For the two cruises during which total length did not differ with distance from shore, age also did not differ with distance from shore (15–17 May, multiple regression: $n = 89$, $r^2 = 0.04$, $P = 0.81$ for distance from shore, $P = 0.23$ for region, $P = 0.91$ for the interaction; 30 May, linear regression: $n = 20$, $r^2 = 0.02$, $P = 0.51$) (Fig. 4).

The offshore distribution for yolk volume and number of caudal fin rays support the trends observed for age. When age decreased with distance from shore on 21 May, there was a tendency for offshore larvae to have greater yolk volumes (multiple regression: $n = 129$, $r^2 = 0.13$, $P = 0.001$ for distance from shore, $P = 0.41$ for region, $P = 0.16$ for the interaction) and fewer caudal fin rays (multiple regression: $n = 129$, $r^2 = 0.12$, $P = 0.0004$ for distance from shore, $P = 0.96$ for region, $P = 0.32$ for the interaction) (Fig. 4). The same was also true for the other cruise with an offshore age trend, 19–20 June, for number of caudal fin rays (linear regression: $n = 21$, $r^2 = 0.19$, $P = 0.05$), but not for yolk volume (linear regression: $n = 22$, $r^2 = 0.04$, $P = 0.36$), although very few larvae still had yolks at this point in the season. Conversely, cruises that did not show an offshore trend for age also did not show an offshore trend for yolk volume (15–17 May, multiple regression: $n = 99$, $r^2 = 0.04$, $P = 0.23$ for distance from shore, $P = 0.10$ for region, $P = 0.09$ for the interaction; 30 May, linear regression: $n = 20$, $r^2 = 0.04$, $P = 0.41$) or for number of caudal fin rays (15–17 May, multiple regression: $n = 98$, $r^2 = 0.06$, $P = 0.46$ for distance from shore, $P = 0.26$ for region, $P = 0.95$ for the interaction; 30 May, linear regression: $n = 20$, $r^2 = 0.01$, $P = 0.62$) (Fig. 4).

Hatching period of cisco

Captured cisco were estimated to have hatched from 22 March to 17 May, with the bulk of the hatch occurring from mid-April to early May. Mean hatch dates increased with cruise for both Ontonagon (single-factor ANOVA:

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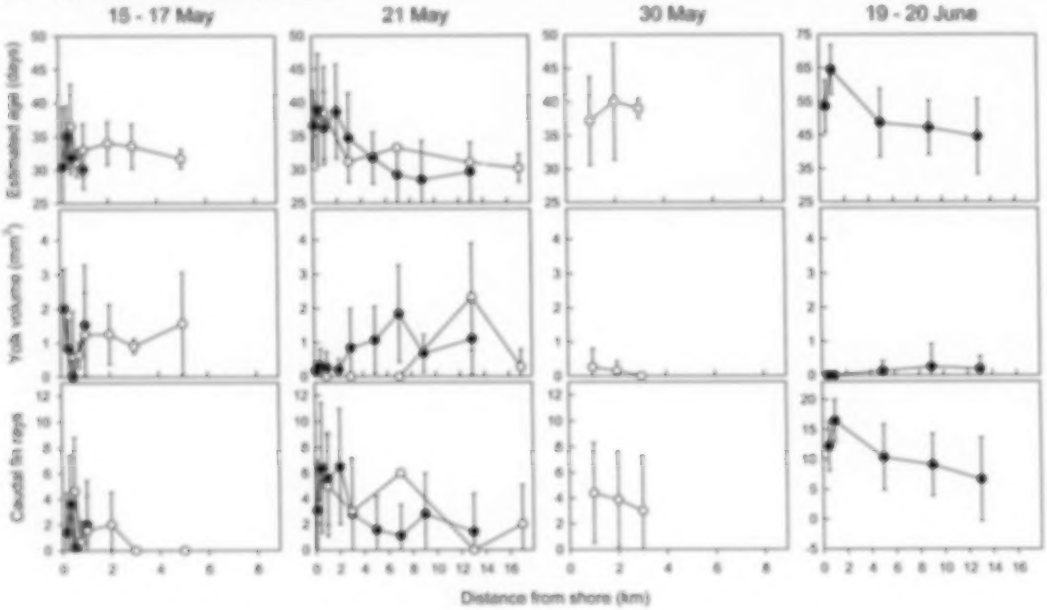
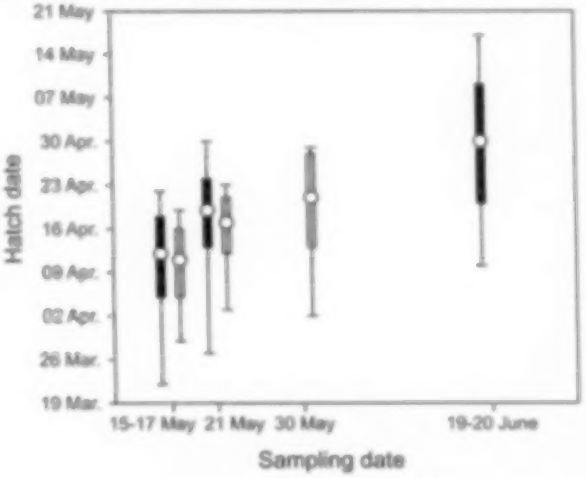
Fig. 4. Offshore trends in mean (± standard deviation, SD) age, yolk volume, and number of caudal fin rays of larval cisco (*Coregonus artedi*) collected during four sampling cruises in 2000 from the Ontonagon (●) and Houghton (○) transects in Lake Superior off the western coast of the Keweenaw Peninsula, Michigan.



$F_{[2,195]} = 55.68$, $P < 0.0001$) and Houghton (single-factor ANOVA: $F_{[2,99]} = 27.12$, $P < 0.0001$), whereby for both regions, each cruise was statistically different from each other (Tukey's test: least significant comparison, $P < 0.001$ for Ontonagon, $P < 0.05$ for Houghton) (Fig. 5). Correspondingly, the ranges of hatch dates tended to shift toward more recent dates with subsequent cruises (Fig. 5), meaning that early-hatch larvae did not exist in later catches, and late-hatch larvae did not exist in earlier catches, even when most of the late-hatch larvae hatched prior to the earlier sampling dates.

Fig. 5. Hatch date of larval cisco (*Coregonus artedi*) collected during four sampling cruises in 2000 from the Ontonagon (solid bars) and Houghton (open bars) transects in Lake Superior off the western coast of the Keweenaw Peninsula, Michigan. Open circles represent the mean, bar limits represent the standard deviation, and whiskers represent the range in hatch dates.



Larval fish transport

Both the higher concentration of younger larvae farther offshore at Ontonagon and the increase in mean hatch date with subsequent sampling cruises could have resulted from larvae being transported through the study region by prevailing currents. Further evidence for larval fish transport was investigated with plots of the percentages of larvae from different hatch periods (early-, bulk-, and late-hatch) for each transect division (0.1–2 km, 3–5 km, and 7–17 km from shore), region (Ontonagon and Houghton), and sampling cruise (Fig. 6). We observed that during 15–17 May, early-hatch larvae composed a small percentage of the inshore catch and late-hatch larvae were not present. During the next cruise, 21 May, early-hatch larvae were still concentrated inshore, and now few late-hatch larvae were encountered offshore, but only at Ontonagon. Late-hatch larvae were encountered at Houghton on 30 May, and by 19–20 June, late-hatch larvae composed approximately half of the catch at Ontonagon, with early-hatch larvae being completely absent in the samples. These patterns show that larvae did not arrive and depart the study region from random locations, but rather followed the flow of the Keweenaw Current.

Spatial and hatch date differences in growth rates

A length-to-age linear regression for all captured larvae showed an average (± standard error, SE) growth rate of 0.18 (±0.008) mm·day^{-1} (Fig. 7). First, this overall growth

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Fig. 6. Percentage of larval cisco (*Coregonus artedi*) from each hatch period for each sampling cruise in 2000, transect division, and region in Lake Superior, off the western coast of the Keweenaw Peninsula, Michigan. Hatch periods were 22 March – 8 April for early-hatch (open fill), 9 April – 27 April for bulk-hatch (hatched fill), and 27 April – 17 May for late-hatch (solid fill). Sample sizes are noted above each bar.

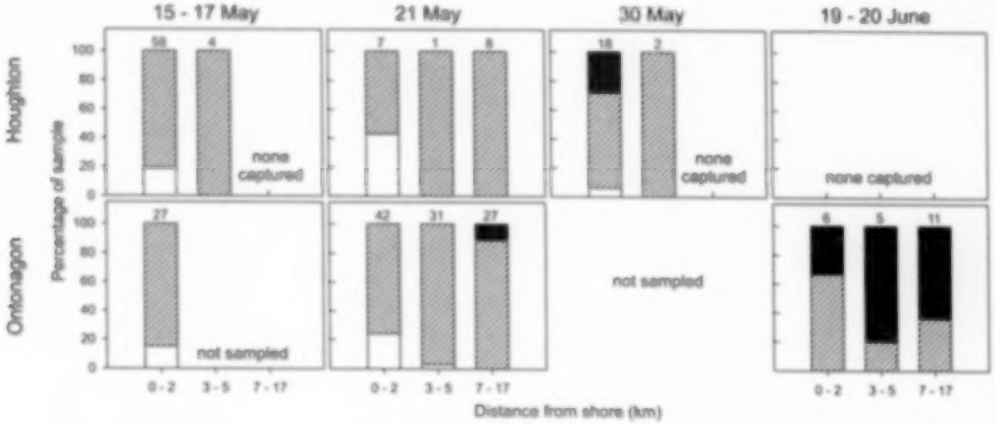


Fig. 7. Total length (TL) to age relationship of larval cisco (*Coregonus artedi*) collected in 2000 from Lake Superior off the western coast of the Keweenaw Peninsula, Michigan. The slope of the linear regression indicates the overall daily growth rate.

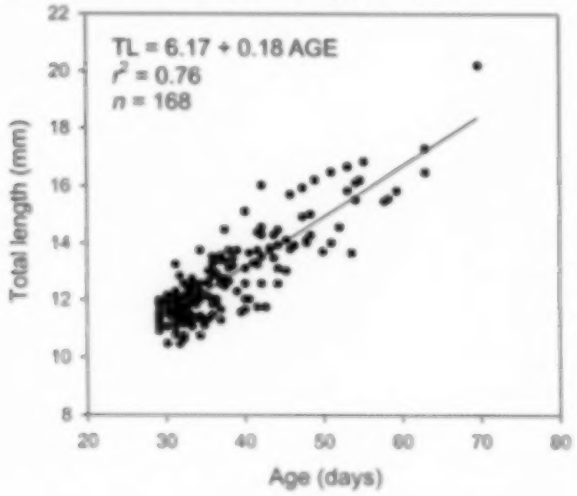
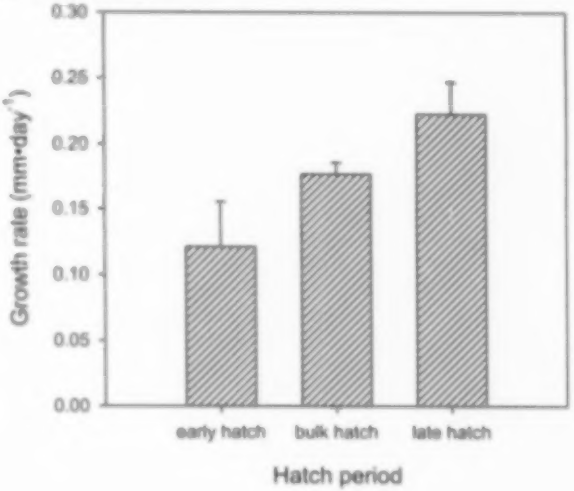


Fig. 8. Growth rates (+ standard error, SE) of larval cisco (*Coregonus artedi*) that hatched during the different hatch periods (early-hatch = 22 March – 8 April, bulk-hatch = 9 April – 27 April, and late-hatch = 27 April – 17 May). Growth rates were estimated as the slope of length-to-age linear regressions for larvae collected in 2000 from Lake Superior off the western coast of the Keweenaw Peninsula, Michigan.



rate was separated by larvae of different hatch periods to determine if late-hatch larvae grew faster because they hatched into, and spend more of their lives in, warmer waters. A multiple regression model for total length as the dependent variable, with age, hatch period, and the interaction as independent variables, showed that the length-to-age relationship was statistically affected by hatch period (n = 168, r^2 = 0.77, P = 0.03 for hatch period × age interaction). By estimating a separate linear regression for each hatch period, we see that the slope (growth rate) does in fact increase with subsequent hatch periods, as expected (Fig. 8), but only slopes for the early and late hatch periods were statistically different from each other (Tukey's test, $P < 0.01$).

Second, the overall grow rate was separated by larvae of different spatial locations (regions and distances from shore) to determine if warmer waters inshore, especially at Ontonagon, fostered faster growth. A multiple regression model (n = 168, r^2 = 0.78) for total length as the dependent variable, with age, region, distance from shore, and all interactions as independent variables, showed that the length-to-age relationship was statistically affected by distance from shore (P = 0.02 for distance from shore × age interaction) but not by region (P = 0.19 for region × age interaction); however, the effect with distance from shore differed by region (P = 0.01 for region × distance from shore × age interaction). Growth rates were calculated directly from the data as slopes of separate linear regressions for regions, with adjacent dis-

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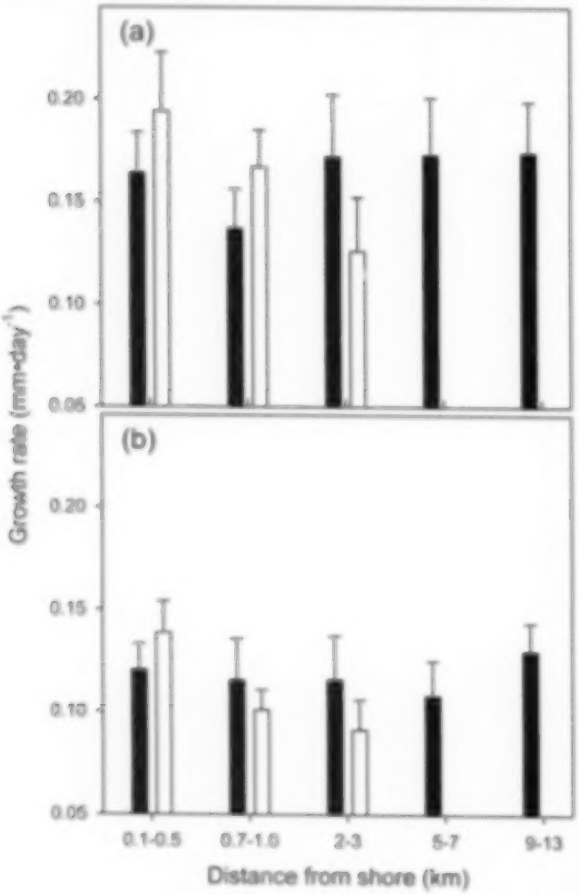
Fig. 9. Spatial differences in growth rates of larval cisco (*Coregonus artedi*), where growth rates were calculated by (*a*) length-to-age linear regressions (slope ± standard error, SE) and by (*b*) back-calculating growth for the last 5 days before capture (mean ± SE). Cisco were collected in 2000 from different distances from shore at Ontonagon (solid bars) and Houghton (open bars) in Lake Superior off the western coast of the Keweenaw Peninsula, Michigan.



tances from shore pooled to achieve sufficient sample sizes (Fig. 9). Estimated slopes (growth rates) decreased with distance from shore at Houghton, but not at Ontonagon (Fig. 9). Furthermore, when estimating models separately by region, an effect for distance from shore occurred at Houghton (multiple regression: $n = 84$, $r^2 = 0.67$, $b = -0.036$, and $P = 0.006$ for distance from shore × age interaction), but not at Ontonagon (multiple regression: $n = 84$, $r^2 = 0.79$, $b = 0.00024$, and $P = 0.93$ for distance from shore × age interaction).

Spatial differences in growth can similarly be assessed using estimated growth rates for the last 5 days before capture. This measure of growth more likely represents the habitat a larva was captured from, considering that cisco have the potential to travel great distances during their early lives. A similar multiple regression model ($n = 88$, $r^2 = 0.18$), including age as a covariate, showed an effect for distance from shore ($P = 0.04$) and not for region ($P = 0.11$); however, region interacted with distance from shore ($P = 0.04$), as would be expected based on the previous growth results. A similar trend was observed graphically with plotted mean growth rates (Fig. 9), in which rates of recent growth decreased with distance from shore at Houghton, but not at Ontonagon. Separate analyses by region confirmed that the recent growth rate was statistically affected by distance from shore at Houghton (multiple regression: $n = 37$, $r^2 = 0.24$, $b = -0.024$, and $P = 0.03$ for distance from shore), but not at Ontonagon (multiple regression: $n = 51$, $r^2 = 0.13$, $b = -0.00002$, and $P = 0.99$ for distance from shore).

Relationship between growth rates and water temperatures

The spatial trend in growth rates at Houghton intuitively followed patterns of water temperatures, but further investigation was required to explain why such a trend was not seen at Ontonagon, where water temperatures also decreased with distance from shore. By regressing the length-to-age relationship on surface water temperature instead of distance from shore, it was observed that the growth rate decreased with decreasing temperatures more strongly at Houghton (multiple regression: $n = 84$, $r^2 = 0.65$, $b = 0.025$, and $P = 0.09$ for temperature × age interaction) than at Ontonagon (multiple regression: $n = 84$, $r^2 = 0.81$, $b = 0.017$, and $P = 0.01$ for temperature × age interaction), although strangely, the effect was more significant at Ontonagon. The same trend was observed for recent growth rates, but with less statistical significance, in which Houghton (multiple regression: $n = 37$, $r^2 = 0.15$, $b = 0.008$, and $P = 0.28$ for temperature) showed a stronger effect over Ontonagon (multiple regression: $n = 51$, $r^2 = 0.13$, $b = 0.00036$, and $P = 0.96$ for temperature). These trends can be visualized by plotting the residuals of length vs. age and recent growth rate vs. age against water temperatures, so that the data are standardized for differences in age (Fig. 10). From this figure it can be seen that (*i*) Houghton and Ontonagon contained different ranges of temperatures and (*ii*) growth rate tended to decrease with temperature at the colder temperatures observed at Houghton and was unaffected by temperature at the generally warmer temperatures observed at Ontonagon.

Finally, the data set was divided, not by region as done previously, but by proposed threshold temperatures, below which temperature was expected to inhibit growth relative to growth above the proposed threshold. The greatest effect of temperature was below a proposed threshold of 8.5 °C with the length-to-age approach to estimating growth rate (multiple regression: $n = 128$, $r^2 = 0.65$, $b = 0.027$, and $P = 0.02$ for age × temperature interaction) and below 8 °C with the back-calculated approach (multiple regression: $n = 45$, $r^2 = 0.08$, $b = 0.013$, and $P = 0.11$ for temperature) (Fig. 11). But overall there was still great variation in growth rates at given temperatures, even after the data were standardized for differences in age.

Discussion

The distribution patterns of cisco followed what would be expected if larvae were being transported by currents from productive spawning regions in western Lake Superior (Goodyear et al. 1982). It is also possible that some larvae originated from historic spawning grounds from within the

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Fig. 10. Relationship between growth rate of larval cisco (*Coregonus artedi*) and surface water temperature. Cisco were collected in 2000 from different distances from shore at the Ontonagon (●) and Houghton (○) transects in Lake Superior off the western coast of the Keweenaw Peninsula, Michigan. Growth rates were estimated by (*a*) length-to-age (TL, total length) linear regressions and by (*b*) back-calculating growth for the last 5 days before capture for each region. The effect of age was standardized by presenting the residuals of each model. Residuals were calculated separately for each region.

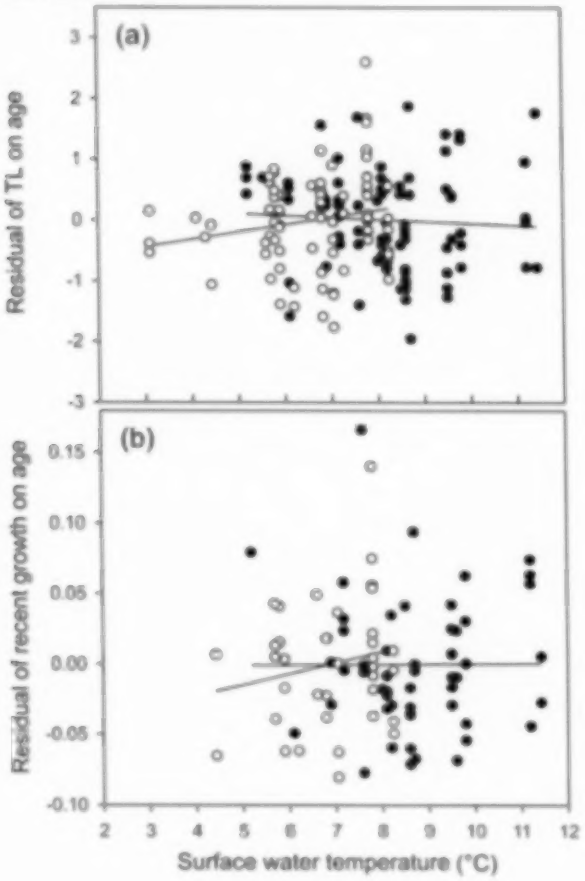
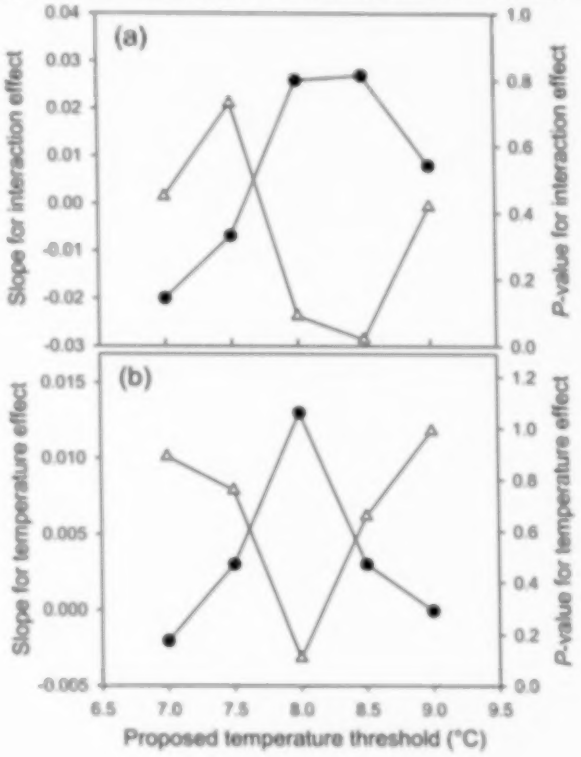


Fig. 11. Regression coefficients (slopes, ●) and *P* values (△) for linear regression models below proposed threshold temperatures. For (*a*) regression-estimated growth, slopes and *P* values are for the age × temperature interaction; for (*b*) back-calculated recent growth, slopes and *P* values are for the temperature main effect. Age was also included in the back-calculated model as a covariate.



study area, at either Ontonagon or Houghton. It was expected that larvae would be more abundant nearer to spawning grounds and less abundant as they disperse away from them. In fact, the greatest densities were observed far offshore at Ontonagon, and Ontonagon generally had higher densities than Houghton. The high-density patch encountered at 7–13 km from shore at Ontonagon consisted of larvae that were younger than those captured nearer to shore. However, these offshore larvae did not hatch recently, but instead averaged slightly less than 30 days of age. Therefore, even if the offshore larvae were encountered at a high concentration akin to a hatching location, these larvae also had sufficient time to travel from some distant location.

The progression of hatch dates with subsequent cruises also supports the conclusion that cisco were transported eastward, following the general flow of currents. The lack of early-hatch larvae in samples from later cruises can be explained by larvae being transported from the study area or by the following alternative mechanisms. First, because early-hatch larvae would have to survive for a longer duration to still exist during later dates compared with recently hatched larvae, a greater cumulative mortality for early-hatch larvae could account for their absence at later dates (Campana and Jones 1992). Second, early-hatch larvae may have grown beyond the size range for which they are susceptible to capture with ichthyoplankton nets (Karjalainen and Viljanen 1992). In contrast, the lack of late-hatch larvae in samples from earlier cruises can not be explained by these alternative mechanisms. The late-hatch larvae would have been more susceptible to capture by plankton nets and would have undergone less cumulative mortality on earlier dates than on later dates. Therefore, the presence of late-hatch larvae only on the later sampling dates, in conjunction with an increase in mean hatch date with subsequent cruises, further supports the conclusion that larvae were transported from adjacent regions. In addition, a cruise covering Ontonagon and Houghton on 27–29 April captured only two larvae with 2715 m^3 of water sampled even though this cruise occurred during the peak of the hatch.

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The spatial patterns of larvae of different hatch periods point to offshore Ontonagon as the location where late-hatch larvae first arrived in the study region. From there, they appeared to have dispersed inshore and up the Keweenaw coast to Houghton. In addition, early-hatch larvae were concentrated inshore during earlier cruises and were rare or absent on later cruises. Therefore, it appeared that cisco moved progressively through the study region, and by the time the late-hatch larvae arrived in inshore areas, early-hatch larvae were almost absent.

Wind-driven currents have been found to be important for transporting fish larvae in many large aquatic systems (Werner 2002). For example, Fechhelm and Griffiths (1990) studied the 600–700 km westward transport of Arctic cisco (*Coregonus autumnalis*) larvae along the Alaskan and Canadian Beaufort Sea coast. They found that years of moderate to high recruitment were associated with strong easterly winds during summer, and years of poor recruitment occurred when easterly winds were weak. Anomalous winds can also disrupt a transport system, as was documented by Bailey et al. (1999) for walleye pollock (*Theragra chalcogramma*) larvae in Shelikof Strait, Gulf of Alaska, during 10 days in May 1996. Recently, the offshore transport of yellow perch (*Perca flavescens*) larvae in Lake Michigan has been documented by Dettmers et al. (2005) and modeled by Beletsky et al. (2007). Fishes may even be adapted to spawn at particular locations and times so to better ensure that their eggs or larvae will be successfully transported to nursery grounds (Parrish et al. 1981).

Once in the study region, cisco tended to congregate at Ontonagon and at inshore areas at both Ontonagon and Houghton. The larvae persisted at Ontonagon later in the season than at Houghton, but if they were only drifting passively through the study region, Houghton should have contained the last of the larvae. Likewise, inshore areas contained a concentration of older larvae that unlikely would have formed and persisted if larvae were only passive drifters. They seemed to be able to remain in the study region for a longer duration when at inshore locations or at Ontonagon, where they likely avoided most of the influence of the Keweenaw Current, compared with larvae at farther distances from shore or farther along the Keweenaw coast where waters deepen and the current intensifies (Niebauer et al. 1977; Green and Terrell 1978). In fact, larvae at Houghton on 5–7 June were rarely encountered, despite extensive sampling from both north and south transects from distances of 0.5–7.0 km from shore, and during the last cruise, Houghton samples were completely devoid of cisco. Therefore, after a period of dispersal, cisco appeared to have settled into nearshore areas where they probably had more control of which habitat to occupy, and more larvae remained near Ontonagon.

Otolith analysis provided estimates of hatch dates and growth rates directly from age, which was not previously feasible for cisco without a validation study (Oyadomari and Auer 2007). The estimated hatch dates, primarily from mid-April to early May, corresponded to what was observed previously with distribution data from western Lake Superior (Hatch and Underhill 1988) and from the St. Mary's River (Jude et al. 1998). Estimated mean growth rates of 0.12–0.22 mm·day^{-1} were comparable with a mean estimate of 0.27 mm·day^{-1} for the St. Mary's River (Jude et al. 1998), but were lower than an estimate of 0.69 mm·day^{-1} for western Lake Superior by Hatch and Underhill (1988), although they believed this was an overestimation.

Larval growth rates were compared in order to determine if larvae received some benefit from remaining at inshore locations. It was expected that growth would be higher in the warmer inshore waters, especially at Ontonagon. Morphological characteristics alone indicated that inshore-captured larvae were larger than larvae caught at farther distances from shore, and sampling in this study region during previous years revealed a similar trend (Oyadomari and Auer 2004). But then age explained much of this difference, with inshore larvae being correspondingly older. When analyzing for differences in growth rates, while accounting for the variation in age, only the Houghton catches displayed an offshore trend, in which growth was slower at farther distances from shore. The growth rates of the offshore larvae at Houghton appeared to have been suppressed from what was achieved at Ontonagon.

The difference between regions in the offshore relationship of growth rate could be explained by temperature differences, whereby water temperatures at Ontonagon were usually high enough not to limit growth. In the laboratory, McCormick et al. (1971) observed that cisco growth increased with temperature in the range 3–18 °C; however, mortality started to increase substantially from approximately 13 °C. The final preferendum for cisco is 9–14 °C (Wismer and Christie 1987); thus, it was reasonable to find that temperature inhibited growth below 8 °C. Temperature may also explain the increase in growth rate with later hatch dates. Because of seasonal warming, larvae that hatched later would have spent less time swimming in colder waters. However, there was still great variation in the distributional patterns. Such variation probably resulted from the fact that larvae were moving and that the temperatures at which they were captured may not have represented the predominant temperatures they experienced throughout their young lives.

Water temperature has been shown to be important for growth in other *Coregonus* species as well. Early growth of the common whitefish (*Coregonus lavaretus*) from Lake Constance (Germany) closely tracked spring warming and not zooplankton abundance (Eckmann and Pusch 1989; Rey and Eckmann 1989), and subsequent year-class strength was associated with early thermal stratification (Eckmann et al. 1988). Earlier-hatched bloater (*Coregonus hoyi*) larvae from Lake Michigan grew slower, and consequently suffered greater mortality, probably owing to increased predation (Rice et al. 1987).

At higher temperatures, another factor, most likely food resources, may have limited growth via an increased metabolic demand. Larvae at higher temperatures would have to consume more food to achieve the same growth rate at a lower temperature (Houde 1989). Therefore, at higher temperatures a higher growth rate can only be obtained with an adequate food supply. Increasing metabolic requirements with seasonal warming may explain why larvae probably depart inshore areas by late June to early July (Clady 1976; Hatch and Underhill 1988; Oyadomari and Auer 2004).

With faster growth within a suitable habitat, cisco larvae would have a greater probability of survival. In general, lar-

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vae that obtain larger sizes have greater swimming abilities, are more successful at feeding, are more resistant to starvation, and are less susceptible to predators (Miller et al. 1988; Jones 2002). Larger-sized cisco in particular have been shown to better survive a simulated winter (Pangle et al. 2004). Because of the lack of a sufficient number of older larvae, size-selective mortality could not be evaluated with otolith analysis for cisco, but mortality in many species has commonly been shown, with otolith analysis, to be size dependent, whereby smaller larvae or juveniles suffer greater mortality (e.g., Post and Prankevicius 1987; Meekan et al. 1998; Gronkjaer et al. 2004).

A strong year class requires successful reproduction, followed by high survival of eggs, larvae, and juveniles (Houde 1987). It is only after hatching that offspring have some control over their survival. Larvae can increase their probability of survival by inhabiting areas that foster faster growth and contain fewer predators (Simpson 1987). Reaching such areas, however, may be impossible for larvae with limited swimming abilities if it were not for the aid of longshore currents. The wind-driven currents in Lake Superior have potential to disperse larvae away from spawning concentrations and into adjacent areas. If larvae are not dispersed, they may suffer greater mortality by attracting predators or by competing for limited food resources. Because of its large size, Lake Superior appears to behave more like an ocean than a lake. Accordingly, the role of meteorological and hydrodynamic processes controlling transport should be explored by recruitment studies of fishes with pelagic larvae, such as cisco.

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Uncertainties in population dynamics and outcomes of regulations in sockeye salmon (*Oncorhynchus nerka*) fisheries: implications for management

Carrie A. Holt and Randall M. Peterman

Abstract: Fisheries managers usually have multiple options available but are often unclear on how to choose among them owing to uncertainties in biological and management components of fisheries systems. We evaluated the performance of current and possible future assessment and management practices for sockeye salmon (*Oncorhynchus nerka*) in British Columbia and Alaska by using a computer model that included major biological and management components and their associated uncertainties (interannual variability in recruitment, age-at-maturity, and sex ratio, as well as uncertainty in observations of spawner abundances, forecasts of recruitment, and outcomes from implementing management regulations). One option for management practices that we evaluated was designed to make the forecasting model more realistic by accounting for long-term trends in age-at-maturity. A second option was designed to reduce deviations between management targets and actual or "realized" harvest levels. We found that compared with practices that ignore those sources of uncertainty, the second option produced annual catches that were higher, on average, and less variable over time while maintaining recruitment above critical conservation levels. Contrary to our expectations, the first modification did not result in comparable benefits. Our results demonstrate the value of using simulation models to evaluate potential modifications to Pacific salmon management practices.

Résumé : Les gestionnaires de la pêche se retrouvent fréquemment face à plusieurs marches à suivre possibles, mais ne voient souvent pas clairement comment choisir entre elles, à cause des incertitudes qui existent au sujet des composantes de biologie et de gestion dans les systèmes de pêche. Nous évaluons la performance des pratiques présentes et des pratiques futures potentielles d'évaluation et de gestion du saumon rouge (*Oncorhynchus nerka*) en Colombie-Britannique et en Alaska à l'aide d'un modèle informatique qui comprend les principales composantes de la biologie et de la gestion ainsi que leurs incertitudes associées (la variabilité d'une année à l'autre du recrutement, de l'âge à la maturité et du rapport mâles–femelles, de même que les incertitudes des observations des abondances de reproducteurs, des prédictions du recrutement et des effets de la mise en vigueur des règlements de gestion). L'une des modifications possibles de gestion que nous avons examinées a été calculée pour rendre le modèle prédictif plus réaliste en tenant compte des tendances à long terme de l'âge à la maturité. Une seconde modification visait à réduire les déviations entre les cibles de gestion et les niveaux actuels ou « réalisés » de la récolte. Nous observons que, par comparaison avec les pratiques de gestion qui ignorent ces sources d'incertitude, la seconde option donne des récoltes annuelles qui sont en moyenne plus élevées et moins variables dans le temps, tout en maintenant le recrutement au-dessus des niveaux critiques pour la conservation. Contrairement à nos attentes, la première modification ne génère pas d'avantages comparables. Nos résultats démontrent l'avantage d'utiliser des modèles de simulation pour évaluer les modifications potentielles des pratiques de gestion des saumons du Pacifique.

[Traduit par la Rédaction]

Introduction

Uncertainties, which are pervasive in biological, human, and management components of fisheries systems, arise because of variability within each component and complex interactions among components (Peterman 2004). The consequences of proposed management actions are usually unclear because of these uncertainties, which are often only incompletely considered by stock assessment scientists when evaluating the effects of proposed management actions. Particularly neglected are uncertainties in both long-term trends in life history characteristics and accuracy with which management targets are typically achieved.

"Management procedures" are sets of rules used by man-

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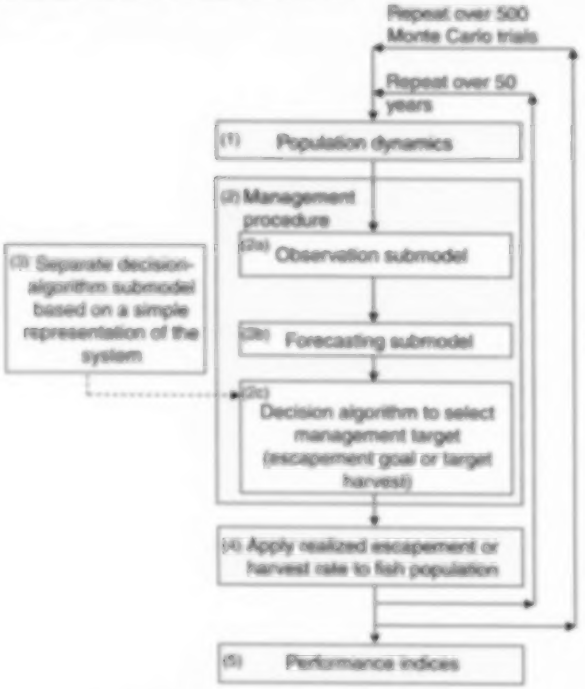
Fig. 1. Simplified outline of the simulation model used to evaluate management procedures for sockeye salmon (*Oncorhynchus nerka*) fisheries in British Columbia and Alaska.


Repeat over 500 Monte Carlo trials
Repeat over 50 years
(1) Population dynamics
(2) Management procedure
(2a) Observation submodel
(2b) Forecasting submodel
(2c) Decision algorithm to select management target (escapement goal or target harvest)
(3) Separate decision-algorithm submodel based on a simple representation of the system
(4) Apply realized escapement or harvest rate to fish population
(5) Performance indices

agers that specify how data are collected on the state of a fishery, how population status is assessed, and how decisions are made (Butterworth et al. 1994; Cochrane et al. 1998). We would expect management procedures that account for uncertainties to better achieve management objectives than those that do not. However, such improvement is not guaranteed. Feedback loops and interactions among system components may offset some of the benefits. Furthermore, derivations of management procedures have to rely on imperfect models of the real world when identifying appropriate decision-making algorithms.

A practical approach to evaluating management procedures is to simulate an entire fisheries system in a computer model ("operating model") that includes biological, human, and management components (i.e., "management strategy evaluation"; Sainsbury et al. 2000) (Fig. 1). This approach differs from traditional simulation modelling commonly used in stock assessment because it incorporates most known major components of the system and their associated uncertainties, including biological processes, surveys of abundance, scientific assessment of a stock, subsequent management actions, responses of harvesters to those actions, and effects on fish population dynamics. Furthermore, these operating models attempt to reflect reality by basing choices of management actions on estimates of the "true" population (i.e., from simulated surveys of abundance), but in contrast to the real world, it is possible to compare the simulated "true" abundance in a computer model with the simulated estimate of abundance. When management actions are evaluated prospectively in this way, the degree to which objectives are achieved can therefore be identified and the relative performances of different management procedures can be assessed (de la Mare 1996).

Fisheries managers often have many options for changes to current management practices. These options usually pertain to different steps in a management procedure. For example, recent evidence suggests that forecasting accuracy might be improved by newly developed forecasting models that account for uncertainty in age-at-maturity of sockeye salmon (*Oncorhynchus nerka*) (Holt and Peterman 2004). Furthermore, harvest rules that account for the commonly observed types and magnitudes of uncertainty in outcomes from implementing fishing regulations, i.e., outcome uncertainty, may improve the accuracy with which management targets are achieved for sockeye salmon (Holt and Peterman 2006). A simulated comparison of practices that use these modifications with practices that ignore them (i.e., the base case) may help scientists and managers focus efforts on actions that will best achieve management objectives.

Our research objective was to use computer simulations to evaluate the respective benefits of two modifications to the base case, one that improved realism (and potentially the accuracy) of one type of forecasting model and another that took into account outcome uncertainty for sockeye salmon fisheries in British Columbia and Alaska. This paper emphasizes a more comprehensive method for evaluating performance of different modifications to management practices for Pacific salmon than is normally the case. Our two examples of salmon populations demonstrate the method rather than make recommendations for specific practices to be applied to particular fisheries on sockeye salmon.

More specifically, the first modification to the base case (i.e., the procedure that ignored those two sources of uncertainty) pertained to forecasts of recruitment of sockeye salmon prior to the fishing season (the number of adults that return to freshwater every year and become vulnerable to the fishery). Forecasting models such as the "sibling model" (Peterman 1982; Wood et al. 1997) are used by industry to help prepare for the fishery and by management to set early-season regulations. We developed a new Kalman-filter version of this model that in a retrospective analysis, improved forecasts for some stocks by accounting for long-term time trends in age-at-maturity (Holt and Peterman 2004). However, any tendency to improve achievement of management objectives (e.g., escapement goals or the target number of spawners) owing to more accurate forecasts could be swamped by large natural variation in recruitment or deviations between management targets and outcomes from fishing regulations (outcome uncertainty), negating the usefulness of this new forecasting model. In other words, it is not clear whether, in practice, this modification to the forecasting method will help managers more accurately achieve management objectives when that method is embedded in a larger fisheries system with its inherent complexity.

Our second modification to the base case pertains to how target escapements or target harvest rates are identified by stock assessment scientists. One way to set a target is to simulate the fisheries system in a simple computer model and use an optimization routine to search for the escapement goal or state-dependent harvest rule (e.g., Fig. 2) that best

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achieves a given management objective. However, the fact that actual harvest levels often deviate substantially from targets (Bocking and Peterman 1988; Eggers 1992; Holt and Peterman 2006) is rarely accounted for when comparing performances of various escapement goals or harvest rules in these types of simulation models (McAllister et al. 1999; Kell et al. 2005). Such deviations (outcome uncertainties) arise from, for example, incomplete compliance by harvesters and incomplete control over outcomes by management agencies after issuing fishing regulations ("implementation uncertainty" or "implementation error"; Rosenberg and Brault 1993; Kell et al. 1999), temporal variability in catchability of fish (Cass et al. 2003), and numbers of fishing vessels, as well as errors in estimating fish abundance (Rosenberg and Restrepo 1994). Intuitively, we might expect that management performance could be improved by taking these factors into account prior to setting fishing regulations (e.g., by appropriately adjusting escapement goals or harvest rules). However, again the extent to which improvements are possible is unknown because of the system's complexity and feedback loops. Thus, to clarify the relative merits of these two modifications to the base case, we conducted simulations to determine the net effect of accounting for uncertainties in age-at-maturity in the forecasting model and outcomes of fishing regulations.

Materials and methods

Data

To parameterize the simulation model for evaluating management procedures, we used previously compiled data on age-specific escapement and total recruitment for two sockeye salmon stocks: Togiak River, Bristol Bay, Alaska (brood years 1952–1998) (Michael Link, LGL, Alaska Research Associates, 1101 East 76th Avenue, Anchorage, AK 99518, USA, personal communication), and Chilko Lake, Fraser River (part of the summer run-timing group), British Columbia (brood years 1948–1998) (Michael LaPointe, Pacific Salmon Commission, 600 – 1155 Robson Street, Vancouver, BC V6E 1B5, Canada, personal communication). For Togiak, estimates of recruitment included the sum of spawning escapement (estimated visually from counting towers) and catch from commercial, recreational, subsistence, personal-use, and test fisheries (Plotnick and Eggers 2004). For Chilko, estimates of recruitment included the sum of spawning escapement (estimated from mark–recapture studies), catch from marine and in-river commercial and test fisheries, Fraser River First Nations harvests, and recreational fisheries, as well as estimates of en-route mortality (Pacific Salmon Commission 2001). Parameters for observation errors in escapement were estimated from repeated tower counts in Bristol Bay, Alaska (2004–2005) (Tim Baker, Alaska Department of Fish and Game, 333 Raspberry Road, Anchorage, AK 99518-159, USA, personal communication), and from unpublished mark–recapture studies in the Fraser River, British Columbia (2001–2004) (Timber Whitehouse, Fisheries and Oceans Canada, B.C. Interior Area Science Branch, 985 McGill Place, Kamloops, BC V2C 6X6, Canada, personal communication). Parameters for the outcome-uncertainty component were estimated from empirical records of target and actual ("realized") escapements for the Togiak River (1962–2004) and from target and realized harvest rates for Chilko Lake (estimated from harvest rates on the summer run-timing group of Fraser River sockeye salmon, 1986–2003) (Holt and Peterman 2006). For Chilko sockeye only, we used previously compiled data on the proportion of spawners that were effective females (1948–1998) (Michael LaPointe, personal communication) to convert total spawner abundance to effective female spawners, as is the standard practice for Fraser River sockeye salmon.

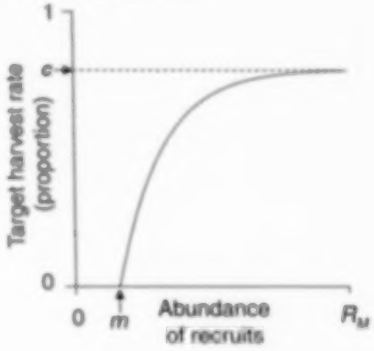

Fig. 2. Example of a harvest rule used to identify target harvest rates for sockeye salmon (*Oncorhynchus nerka*) fisheries in the Fraser River, British Columbia. Parameter m is the protection level in abundance of recruits below which target harvest rate is zero, c is the maximum target harvest rate at high recruitment, and R_M is the maximum observed recruitment. In many salmon fisheries (depending on the management objective), m is the escapement goal, or target number of spawners.

Current management approaches differ for these two stocks. Togiak sockeye salmon are managed for an escapement goal and Chilko sockeye salmon are managed, in part, using a harvest rule (as in Fig. 2). Therefore, the simulation models used to evaluate management options for these populations also differed.

Simulation model for evaluating management procedures

The simulation model used to evaluate management procedures had five components that simulated different aspects of the fishery: population dynamics, the management procedure (which contained an observation submodel, a forecasting submodel, and a decision algorithm to select the management target), steps taken to choose the decision algorithm, the effects of harvesting on the population (including outcome uncertainty), and a performance module (Fig. 1). We describe each component (boxes in Fig. 1) in more detail below. All equations are in Appendix A (Table A1).

Population dynamics component

We simulated the dynamics of hypothetical true sockeye salmon populations for 50 years, repeated over 500 Monte Carlo trials to incorporate stochastic variability in recruitment, age-at-maturity, and proportion of escapement that was effective female spawners (the latter for Chilko only) (box 1 of Fig. 1). That number of Monte Carlo trials was

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Table 1. Description of the three management procedures that we evaluated in the simulation model.

	Components of management procedure	
	Forecasting submodel	Decision-algorithm submodel
Base case	Assumes that parameters are constant	Assumes that management targets are achieved exactly
VM procedure	Time-varying parameters accounted for by using a Kalman filter	Assumes that management targets are achieved exactly
OU procedure	Assumes that parameters of the forecasting submodel are constant	Management targets are adjusted for outcome uncertainty

Note: The VM (variable-maturity) procedure is a modification to the base case that accounts for uncertainty in age-at-maturity in the forecasting submodel. The OU (outcome-uncertainty) procedure is a modification to the base case that accounts for outcome uncertainty through a decision-algorithm submodel. The second and third columns describe our modifications to these two components of the management procedure: the forecasting submodel and the decision-algorithm submodel.

necessary to stabilize output metrics recorded in the performance component of the model.

For Togiak, true spawner abundance in a given brood year was used to calculate recruitment resulting from that spawning using a standard Ricker model, which included lognormally distributed, autocorrelated random variation (the latter with a one-year time lag) (Appendix A, Table A1, eq. A1). For this and all subsequent relations, parameters (Appendix A, Table A1, right side of table) were estimated from historical data using maximum likelihood methods, unless stated otherwise.

For Chilko, we used the slightly more complex Larkin spawner–recruit relation (Walters and Staley 1987) because this relation captures this stock's four-year cyclic pattern in abundance of recruits (Appendix A, Table A1, eq. A2). The abundance of the "dominant" line of Chilko Lake sockeye that occurs every fourth year consistently exceeds that of the subsequent "subdominant" line and the following two "off-cycle" lines.

For both Togiak and Chilko simulations, historical recruitment data were used to estimate the mean proportion of fish of a given freshwater age that matured at each ocean age, as well as to estimate a time trend to reflect the simulated rate of increase in mean ocean age observed for these stocks over the last 50 years (Appendix A, Table A1, eq. A3). For example, for the base case for Togiak for the fish that spent two winters in freshwater, our data show that the proportion that returned after only two winters in the ocean instead of three declined from 0.53 to 0.39 from 1952 to 1998 (and the proportion that returned after three ocean winters increased from 0.47 to 0.61). When combined over all age classes, the increase in mean age-at-maturity for Togiak was 0.186 years, but for Chilko (1948–1998), this increase was much smaller (0.049 years). The simulation model also included natural interannual variation in age-at-maturity according to a multivariate logistic distribution (as in Schnute and Richards 1995) (Appendix A, Table A1, eqs. A4 and A5). Finally, for Chilko Lake sockeye salmon, we calculated the proportion of effective female spawners and incorporated interannual variability into that proportion using a beta distribution parameterized using historical spawner data (Appendix A, Table A1, eqs. A6–A8).

Management procedure

Management actions were broken down into three submodels (box 2 of Fig. 1): (2*a*) observations of abundances of recruitment from total catch and surveys of escapement, (2*b*) forecasts of recruitment using the sibling model, and (2*c*) selection of escapement goal or target harvest rate as specified by a decision algorithm. We evaluated three different management procedures in simulations of the fisheries system (Table 1). The first management procedure represented the base case. The second was a modification of the base case in which the forecasting submodel accounted for time-varying parameters associated with age-at-maturity using a Kalman-filter technique (the variable-maturity or VM procedure). The third was another modification to the base case that adjusted management targets for outcome uncertainties (the outcome-uncertainty or OU procedure), rather than assuming that targets were met exactly.

Observation submodel

The observed number of spawners each year was calculated from the "true" (i.e., simulated abundances in the population dynamics component of the simulation model) number of spawners and multiplicative, lognormally distributed random variability (as in Cass et al. 2003) (box 2*a* of Fig. 1; Appendix A, Table A1, eq. A9). The latter random deviations represent both errors in visual observations of spawner abundance and sampling variability. We assumed that catch was known perfectly and that errors due to stock misidentification were negligible for the Togiak and Chilko sockeye stocks, as determined by analyses of scale patterns and locations of catch that are separate from other stocks (T. Baker, Alaska Department of Fish and Game, 333 Raspberry Road, Anchorage, AK 99518-159, USA, personal communication; T. Whitehouse, Fisheries and Oceans Canada, B.C. Interior Area Science Branch, 985 McGill Place, Kamloops, BC V2C 6X6, Canada, personal communication). In addition, we assumed perfect knowledge of fish age and proportion of spawners that was effective females.

To properly parameterize natural process variation in recruitment (ν or γ, depending on the stock, in eqs. A1 and A2 of Appendix A, Table A1) and observation error (κ, eq. A9 of Appendix A, Table A1), we estimated variability from those two specific sources of the total observed variability in recruitment (see Appendix B for more details).

Forecasting submodel

We implemented two different forecasting submodels. For the base case and OU procedure, we applied the widely used standard linear sibling model that assumed constant parameters over time to forecast age-specific recruitment (box 2*b* of Fig. 1 and Appendix A, Table A1, eq. A10). However, for the VM procedure, we modified that standard sibling model

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to account for temporal changes in age-at-maturity using a Kalman-filter estimation scheme (see Holt and Peterman (2004) for a description of these two parameterizations of the sibling model and Appendix A, Table A1, eqs. A10 and A11). To forecast the abundance of the youngest age class for each freshwater age, we simply used recruitment in the previous generation, instead of the sibling model (as in Haeseker et al. 2007). The standard or Kalman-filter models were used to forecast all older age classes.

Selection of escapement goal or target harvest rate

An annual management target was then chosen based on a decision algorithm (i.e., an escapement goal or target harvest rule) (box 2*c* of Fig. 1) that was identified in the decision-algorithm submodel (box 3 of Fig. 1, described below). For Chilko, a management target was identified from a target harvest rule, and for Togiak, an escapement goal was used.

Decision-algorithm submodel

The decision algorithm was selected in a submodel, which was simulated separately from the main model used to evaluate management options (i.e., it was run once for each management procedure and stock in our experimental design) (box 3 of Fig. 1). Specifically, in the decision-algorithm submodel, we simulated the biological system and management actions using various targets to identify the one that best met management objectives among those considered.

For both stocks, we assumed that the objective was to maximize yield while meeting two constraints: allowing no more than a 10% chance of catch dropping below 10% of maximum sustainable yield (MSY) and no more than a 10% chance of spawner abundances dropping below 10% of the spawner abundance associated with maximum recruitment (SMR) in each year. Although the latter reference point is unusual, it is similar to what was used for the Sockeye Salmon Spawning Initiative, a recent project aimed at revising management procedures for Fraser River, British Columbia, sockeye fisheries (Cass et al. 2003). Such management constraints are often not explicitly documented, so our two constraints represent only one example of possible trade-offs between maximizing catch and minimizing population declines.

This decision-algorithm submodel was similar to the overall simulation model, with some simplifying assumptions. These assumptions (described in Appendix C) reflect factors commonly considered by managers when choosing decision algorithms. However, comprehensive quantitative analyses of decision-algorithm submodels, such as ours in box 3 of Fig. 1, are not routinely performed for Fraser River or Bristol Bay sockeye salmon when identifying decision algorithms.

We ran the decision-algorithm submodel twice for each stock. First, we ran it to identify management targets that ignored outcome uncertainty (an escapement goal for Togiak and a state-dependent harvest rule with parameters c, d, and m for Chilko, as shown in Fig. 2) (Appendix A, Table A1, eq. A12). Second, we ran it to identify targets that took outcome uncertainty into account (a revised escapement goal for Togiak and a harvest rule with parameters c^*, d^*, and m^* for Chilko) (Appendix A, Table A1, eq. A13). For Togiak, the decision-algorithm submodel simulated the bias in deviations between realized and target escapement that appears in the historical data. Specifically, preseason forecasting errors were negatively related to deviations between realized and target escapements for that stock (Fig. 3; $p < 0.001$). For example, when preseason forecasts underestimated the actual, realized recruitment (i.e., to the left of 1.0 on the x axis of Fig. 3), more fish returned to spawning grounds than targeted by the escapement goal. Fewer fish escaped the fishery than targeted in only 2 out of 21 years (i.e., 2 points fell below 1.0 on the y axis of Fig. 3). This tendency towards over-escapement arises because of incentives for Togiak managers to meet or exceed the escapement goal (Hilborn 2006). In the decision algorithm that accounted for outcome uncertainty for Togiak sockeye, we used the relation in Fig. 3 and calculated escapement deviations (i.e., $\log_e(E_t/E_t^*)$, where E_t is realized escapement and E_t^* is target escapement) as a linear function of forecasting errors (i.e., $\log_e(R_t^*/R_t)$, where R^* is the forecast of recruitment and R is realized recruitment) plus normally distributed variability (Appendix A, Table A1, eq. A14).

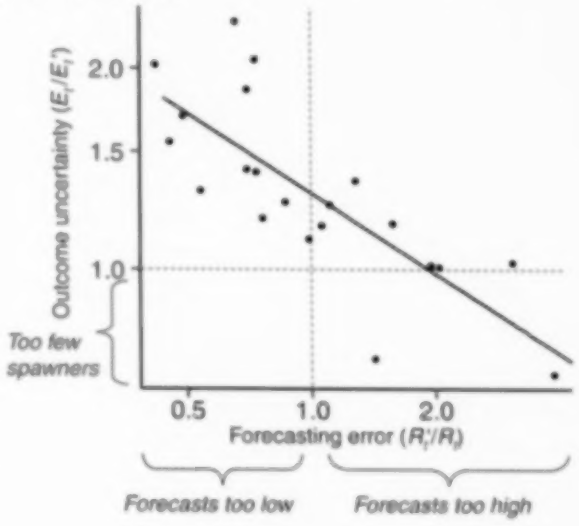

Fig. 3. Relation between annual preseason forecasting error in abundance of adult recruits (R_t^*/R_t) and outcome uncertainty (E_t/E_t^*) plotted on a logarithmic scale, where R_t^* is the forecast of recruitment in year t, R_t is realized recruitment, E_t is realized escapement, and E_t^* is the escapement goal for Togiak River sockeye salmon (*Oncorhynchus nerka*), Bristol Bay, Alaska (1984–2004) ($r^2 = 0.65$, $P < 0.001$). The vertical broken line represents a perfect forecast ($R_t^*/R_t = 1.0$) and the horizontal broken line represents an escapement goal that is achieved exactly ($E_t/E_t^* = 1.0$). This relation is equivalent to that between $[\log_e(R_t^*) - \log_e(R_t)]$ and $[\log_e(E_t) - \log_e(E_t^*)]$.

In contrast, data for Chilko Lake sockeye show no evidence of consistent bias in realized harvest rates compared with targets (as part of the summer run-timing group of the Fraser River; Holt and Peterman 2006), so we simulated imprecision in the outcome from the target harvest rule without bias. Specifically, we included beta-distributed random variability in the realized harvest rate of Chilko sockeye be-

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tween zero and one to simulate imprecision in outcomes (Appendix A, Table A1, eqs. A15–A17).

Harvesting and escapement

In the simulation model's fourth component, we simulated escapement to spawning grounds after harvesting (box 4 of Fig. 1) while adding the effect of uncertainty in outcomes of applying management targets (as described above for the decision-algorithm submodel; Appendix A, Table A1, eq. A14 for Togiak rearranged to solve for realized escapement, E_o, and eqs. A15–A18 for Chilko). For Togiak, that outcome uncertainty was based on the stochastic relation in Fig. 3, which reflects the historical association between outcome uncertainty and forecasting error. We do not imply that this is a direct causal relation, but rather that it is the net result of interactions among accuracy of forecasts of recruitment, the timing and magnitude of in-season updates of abundance, and management actions (Holt and Peterman 2004). Such associations are commonly observed in salmon fisheries. When forecasts overestimate recruitment (which tends to happen at low abundances) (Appendix C, eq. C2), by the time in-season updates are available, it is often too late to restrict harvest pressure to achieve management goals (Bocking and Peterman 1988). In contrast, when forecasts underestimate recruitment, by the time in-season updates are available, it tends to be too late to allow sufficient harvest pressure to achieve goals (Bocking and Peterman 1988). Other sources of uncertainty in outcomes compared with targets are accounted for implicitly by stochastic variability parameterized with historical data. Unlike Togiak, management targets for Chilko are derived from a target harvest rule and forecasts of recruitment, and deviations between management targets and outcomes are not associated with forecasting accuracy. Therefore, forecasts of abundance of adult recruits (not accuracy of those forecasts) were used to identify management targets from the harvest rule for the Chilko stock, and outcome uncertainties were stochastic deviations from that target, parameterized with empirical data (Appendix A, Table A1, eqs. A15–A17).

Historical escapement data that were used to parameterize this component of the model contain observation error as one component of outcome uncertainty. For the overall simulation model that evaluated management options, we separated observation error (as it was already simulated in the observation submodel) using the same method as that used for separating observation error in recruitment from natural variability (see Appendix B).

Performance component

To evaluate how well management procedures achieved objectives, the following metrics were averaged over each 50-year simulation period and over Monte Carlo trials (box 5 in Fig. 1): (1) mean annual catch; (2) proportion of years when catch dropped below 0.1-MSY; (3) proportion of years when escapement dropped below 0.1-SMR (10% of spawner abundance at maximum recruitment); (4) median percent change in catch between successive years; (5) root-mean-square (RMS) forecasting error; (6) mean absolute forecasting error; and (7) mean of the absolute deviations between target and realized escapements or harvest rates.

In addition to those attributes described by the initial management objective (performance metrics 1 through 3), harvesters may prefer reductions in variability of catch among years (captured by metric 4), whereas scientists and managers may be more concerned about forecasting errors (metrics 5 and 6, referred to as RMS forecasting error and bias of forecast, respectively) and deviations between management targets and outcomes (metric 7).

Sensitivity analyses

We evaluated the sensitivity of the rank order of the three management procedures to different assumptions: (*i*) larger increases in age-at-maturity over time than those previously observed in past field data, (*ii*) sinusoidal time trends in age-at-maturity, (*iii*) constant age-at-maturity, and (*iv*) larger magnitudes of observation errors than typically seen for these stocks relative to natural variation in recruitment and outcome uncertainty. We also identified the maximum potential improvement in mean annual catch that would occur over the base case if we assumed that the forecasting model was perfectly accurate.

Finally, we examined the potential consequences of adjusting harvest regulations for outcome uncertainties when such uncertainties do not exist in the "true" population. Specifically, we omitted outcome uncertainty from the simulation model used to evaluate management options and calculated the difference in mean annual catch between a practice that used the OU procedure and the base case that did not. In other words, we evaluated the potential reductions in performance when outcome uncertainty was mistakenly accounted for (when it did not occur in the "true" population), compared with the base case. Although this scenario is unlikely, it provides an upper bound on the magnitude of losses incurred by incorrectly accounting for outcome uncertainty.

Results

We expected that the procedure that accounted for outcome uncertainty when identifying a target escapement or harvest rule (the OU procedure) would have higher mean abundance and annual catch and hence would better achieve management objectives than the procedures that ignored that source of uncertainty. Similarly, because our scenarios assumed long-term trends in age-at-maturity in the "true" population, we expected that when simulated over years and Monte Carlo trials, the management procedure that forecasted recruitment using a sibling model that accounted for those trends in age-at-maturity (the VM procedure) would have lower forecasting errors, improved accuracy of achieving management targets, and therefore higher mean annual catch than the base case that used a standard sibling model (which assumed a constant proportion of maturing fish in each age class).

Togiak

We present results first for Togiak and then for Chilko. As expected, compared with the base case, mean annual catch for Togiak sockeye salmon was higher for the OU procedure by 31 418 fish (11.4%) (Fig. 4*a*). For this OU procedure, the escapement goal (132 660 spawners) was lower than the goal that ignored that source of uncertainty

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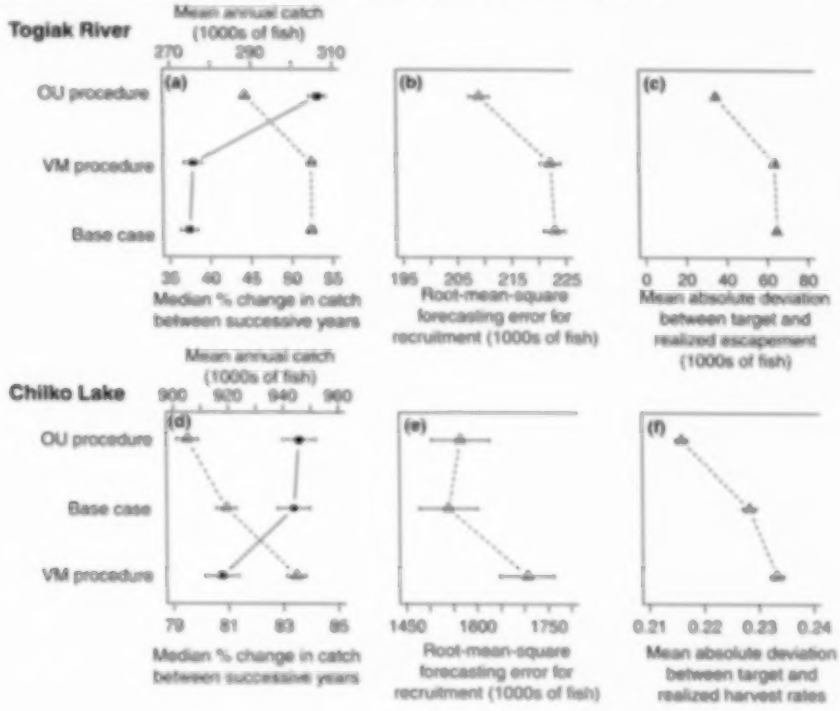
Fig. 4. Trade-offs in performance metrics for (*a*, *b*, and *c*) salmon fisheries on Togiak River, Bristol Bay, Alaska, and (*d*, *e*, and *f*) Chilko Lake, Fraser River, British Columbia, sockeye salmon (*Oncorhynchus nerka*) for three management procedures: outcome-uncertainty (OU) procedure, variable-maturity (VM) procedure, and base case, arranged on the vertical axis according to their rank in mean annual catch (different rankings for Togiak and Chilko). (*a* and *d*) Mean annual catch in 1000s of fish (solid lines and solid squares); median percent change in catch between successive years (i.e., catch variability; broken lines and open triangles); (*b* and *e*), root-mean-square forecasting error for recruitment (i.e., RMS forecasting error); (*c*) mean absolute deviation between target and realized escapement; and (*f*) mean absolute deviation between target and realized harvest rates (proportions). Error bars represent ± one standard error of the mean result across 500 Monte Carlo trials, except in (c) where the error bars are too narrow to distinguish from the mean.



(179 176 spawners); that lower goal compensated for the observed tendency for over-escapement (Fig. 3). Compared with the base case, mean annual catch tended to be higher for the VM procedure, i.e., the one that accounted for long-term trends in age-at-maturity, but this difference was small (943 fish or 0.3% of mean annual catch for the base case). For this and the subsequent five plots in Fig. 4, management procedures are placed on the *y* axis according to their rank in mean annual catch, with the highest on top (Figs. 4*a* and 4*d*). To show trade-offs between mean annual catch and other performance metrics, values for additional metrics are included in Figs. 4*a*–4*f*, scaled according to the bottom *x* axis.

The mean proportion of years when annual catch dropped below 0.1·MSY was consistently low for all three procedures and so is not shown here (<0.07, i.e., less than the 0.1 stated in the management objective). This was also true for the mean proportion of years in which spawning abundance dropped below 0.1·SMR (<0.001, i.e., less than the 0.1 management objective). Because sockeye spawning stocks of Bristol Bay, Alaska, are often compared with their expected spawner abundance at the unfished equilibrium, S_{eq}, we further calculated the mean proportion of years when spawning abundance dropped below $0.1 \cdot S_{eq}$. Again, this value was low for all procedures (<0.001).

For the remaining four performance metrics (interannual variability in catch, RMS forecasting error, bias in forecasts, and deviations between targets and outcomes), the rank orders of management procedures for Togiak were consistent with the rank order for mean annual catch. For example, management procedures that had the highest mean annual catch also had the lowest catch variability (i.e., performance improved on both metrics) (Fig. 4*a*). In contrast to our initial expectations, the RMS forecasting error for Togiak sockeye was not consistently smaller for the VM procedure compared with the base case, even though long-term trends in age-at-maturity occurred in the simulated "true" population (Fig. 4*b*). However, compared with the base case, the RMS forecasting error was consistently smaller for the OU procedure (Fig. 4*b*). Forecasts for the OU and VM procedures were also less negatively biased than the base case and the absolute magnitude of bias was reduced (not shown). As expected, compared with the base case, deviations between target and realized escapement were smaller (performance improved) for the OU procedure (i.e., by 29 856 spawners or 46% of the mean deviation for the base

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case) (Fig. 4*c*). That metric was also lower for the VM procedure compared with the base case, but only by 855 spawners or 1.3% of the mean deviation for the base case.

To summarize results for Togiak, the OU procedure outperformed the base case on all performance measures and either outperformed or performed as well as the VM procedure.

Chilko

Chilko results were qualitatively similar to those of Togiak; mean annual catches were higher for the OU procedure compared with the base case, but this increase was small (1944 fish or 0.2% of mean annual catch for the base case) (Fig. 4*d*). In contrast to our initial expectations, compared with the base case, mean annual catches were lower for the VM procedure (by 25 967 fish, or a 2.8% decrease in mean annual catch).

As was the case for Togiak, the mean proportion of years when annual catch of Chilko Lake sockeye dropped below 0.1-MSY, and the mean proportion of years when spawning abundance dropped below 0.1-SMR, were consistently low for all procedures (<0.07 and <0.06, respectively, i.e., less than the 0.1 in the management objective).

The time series of catches were least variable for the OU procedure, followed by the base case, and VM procedure in order of increasing variability (Fig. 4*d*). However, the range of percent change in catch across those cases was small (<4%). Counter to expectations, the RMS forecasting errors of the VM and OU procedures were larger (i.e., had worse performance) than those errors for the base case (Fig. 4*e*). In contrast, the two modified management procedures had forecasts with smaller positive bias by 4% to 41% compared with the base case (not shown). Deviations between target and realized harvest rates were about 1% smaller for the OU procedure compared with the base case, and the VM procedure was 0.5% greater (Fig. 4*f*).

Although the OU procedure outperformed the VM procedure and the base case on many performance metrics for Chilko, these improvements were small compared with those observed for Togiak (e.g., 1% increase in mean annual catch for the OU procedure over the base case for Chilko compared with an 11% increase for Togiak). The base case outperformed the VM procedure on all performance metrics for Chilko Lake sockeye salmon.

Sensitivity analyses

For both stocks, the rank order of management procedures did not change during our sensitivity analyses. First, there was no change in ranking when evaluating different time trends in age-at-maturity (annual increases twice as large as those observed historically, sinusoidal changes, or no long-term trends). For example, for Togiak sockeye salmon, the relatively small (<1%) increase in catch for the VM procedure over the base-case management procedure occurred regardless of the trends in age-at-maturity in the "true" population. Furthermore, for both stocks, the rank order of management procedures was not sensitive to increases in the magnitude of observation error relative to natural variation in recruitment and outcome uncertainty. When we assumed that the forecasting submodel was perfectly accurate, mean annual catches increased over the base case by 16% and 13% for Togiak and Chilko, respectively.

In a final sensitivity analysis, we asked what would happen if, in the management of the simulated "true" population, targets were achieved exactly, yet the management procedure mistakenly assumed that the target would be missed due to outcome uncertainty and adjusted management actions accordingly. For such cases where management procedures "mistakenly" accounted for outcome uncertainties, the mean annual catches were lower than the base-case management procedure by 2% and 5% for Togiak and Chilko, respectively.

Discussion

Although accounting for key biological and outcome uncertainties in stock assessments may a priori seem justifiable for improving the assessment and management of sockeye salmon, alterations to any one component of our simulated fisheries system did not necessarily enhance performance overall. For example, accounting for outcome uncertainty with the OU management procedure improved management performance, especially for Togiak, but accounting for uncertainty in age-at-maturity in the forecasting submodel with the VM procedure did not. Although our primary interest was to demonstrate a method for evaluating management options, our results suggest that scientists and managers should consider evaluating management practices that adjust management targets to reflect outcome uncertainty instead of (or in addition to) practices that only attempt to improve forecasts of abundance.

Improvements from accounting for outcome uncertainty

Compared with the base case, the management procedure that accounted for outcome uncertainty resulted in larger catches that were less variable from year-to-year for both Togiak and Chilko. This result can be explained for Togiak by the bias that causes realized escapements to exceed targets, as evident in the historical record for that stock. When target escapements are adjusted downwards to compensate for that bias, a larger proportion of the recruitment can be harvested (so mean annual catches increase). Not surprisingly, deviations between target and realized escapements were lower for the OU management procedure compared with the base case. Therefore, by ignoring the historical differences between management targets and outcomes, managers of Togiak sockeye salmon may be inadvertently foregoing some long-term catch. However, if objectives differ from those implied by historical data (e.g., due to incentives by managers to allow over-escapement; Hilborn 2006), then adjustments to the targets such as our simulated ones may be inappropriate. Nevertheless, those deviations should be quantified and explained.

Similarly, for Chilko, the management procedure that accounted for outcome uncertainties was better able to maintain high levels of catch. However, in contrast to Togiak, the outcome of harvest rates for Chilko sockeye was unbiased both in the historical record and in the "true" population in our simulation model of the fisheries system. Thus, improvements in mean annual catch were due only to accounting for the imprecision in achieving target harvest rates, and those benefits were relatively small.

In our base-case analysis, we assumed that the outcomes

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from applying management regulations differed from targets in the "true" population. In the sensitivity analysis, when we instead assumed that outcomes exactly matched targets but managers (incorrectly) accounted for outcome uncertainty, the reduction in mean annual catch over the base case for Togiak (2%) was much less than the increase in catch (11%) when the opposite occurred (i.e., when managers correctly assumed that outcomes differed from targets). However, the opposite was true for Chilko. The losses from mistakenly adjusting target harvest rates (5% reduction in mean annual catch) were larger than the gains associated with adjusting target harvest rates when they were, in fact, implemented with uncertainty in the "true" population (0.1% increase in mean annual catch).

Improvements from accounting for long-term trends in age-at-maturity

For Togiak, the management procedure that accounted for time-varying age-at-maturity (the VM procedure) improved forecasting accuracy only slightly over the base case, and for Chilko, such forecasts were consistently less accurate. The latter inaccurate forecasts were due, in part, to the tendency of the forecasting submodel that used a Kalman filter (the VM procedure) to track random variability in age-specific recruitment ("noise") and the cyclic patterns in Chilko recruitment instead of long-term trends in age-at-maturity, which reflected relatively small changes for that stock. This situation resulted in large interannual variability in parameters of the Kalman-filter version of the forecasting submodel and, hence, relatively inaccurate forecasts of recruitment and reductions in mean annual catch. Furthermore, the rank order of management procedures was independent of long-term trends in age-at-maturity in the "true" population (as shown in our sensitivity analyses), also suggesting that the VM procedure was unable to appropriately capture these trends for Chilko Lake sockeye salmon.

For the standard sibling model for Chilko Lake sockeye, forecasting accuracy improved when outcome uncertainties were ignored (i.e., the base case was better than the OU procedure). This result may be due to increased variability in recruitment and catch, resulting in larger contrast in simulated observed data and therefore better estimates of parameters of the forecasting submodel. However, any increases in such forecasting accuracy for Chilko did not result in increased mean annual catch. For Togiak, management procedures that ignored outcome uncertainties (and reduced management control) did not improve forecasting.

Nevertheless, larger improvements in forecast accuracy with other future modifications to the sibling or other models that were not considered here (e.g., Haeseker et al. 2007) may result in improved mean annual catch. For example, the potential increase in mean annual catch over the base case with a completely accurate forecasting submodel ranged from 13% to 16%. However, given large natural variability in population dynamics, ecological interactions within and among stocks, and effects of changing climate, such large improvements in forecasting accuracy may be unlikely in the foreseeable future.

We also evaluated the performance of a procedure that accounted for both variable maturity in the forecasting submodel as well as outcome uncertainties in the harvest rule or target escapement. Results were dominated by the influence of only one modification, i.e., either the VM or OU procedure, depending on the stock as reported above; the combination did not perform better.

Comparison with previous research: outcome uncertainty

Despite repeated recommendations to incorporate implementation uncertainties (one component of outcome uncertainties) into simulation models when evaluating management options for various fisheries (e.g., Rice and Richards 1996; McAllister et al. 1999), fisheries models that include them are the exception rather than the rule. Previous studies that incorporated outcome uncertainties into simulation models assumed that those differences were either deterministic (i.e., a fixed bias) (Kell et al. 1999) or, if stochastic, that they were unbiased with respect to the target (Johnston et al. 2000). In the few cases where both imprecision and bias have been included, they have been parameterized qualitatively either using only limited empirical data (Kell et al. 1999; Peterman et al. 2000) or expert opinion (Kell et al. 1999). Furthermore, with one exception (Cass et al. 2003), previous studies have not evaluated decision algorithms that specifically account for these deviations. Cass et al. (2003) identified harvest rules for two Fraser River stocks that accounted for uncertainty in achieving management targets by assuming that differences between realized harvest rates and targets were characterized by a random normal deviation around the target, as parameterized by expert judgment. Similar to our results for Chilko, those authors found that accounting for imprecision in this type of uncertainty resulted in relatively small changes in the form of the target harvest rule (Cass et al. 2003). However, in contrast to our study, Cass et al. (2003) took into account only imprecision in outcome uncertainty, and not bias plus imprecision, when evaluating management options.

Comparisons with previous research: evaluating forecasting models

Several studies have quantitatively evaluated the performance of a wide range of forecasting models for sockeye salmon (Wood et al. 1997; Peterman et al. 2000; Haeseker et al. 2007), but none has prospectively evaluated sibling forecasting models that use a Kalman filter, let alone by using a simulation model of an entire fishery system. A common assumption in these other studies is that improvements in forecasting accuracy will result in higher yields and economic gains, but our results show that this is not necessarily the case.

One example of a study that supported the assumption that more accurate forecasts improve management performance used a computer simulation model to evaluate another type of forecasting model, the spawner–recruit relation (Peterman et al. 2000). That study compared parameter estimation methods that did not account for long-term trends in productivity with a Kalman-filter method that did. In contrast to our results, they found that the forecasting model that used a Kalman filter had higher cumulative catches compared with the standard forecasting model. However, Peterman et al. (2000) used a different derivation of out-

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come uncertainty (for both bias and imprecision) than we did in our study. Furthermore, they simulated much larger temporal changes in the variable being estimated (the Ricker *a* parameter) than we did for our variable (the *y* intercept of the sibling model, representing relative ages-at-maturity).

Limitations

Interpretations of our results are affected by at least four limitations. First, the performance of the two modifications to current practices depends on our assumptions about dynamics and structure of the fishery, including assumptions about which components to consider uncertain. For example, for Togiak, we assumed that the realized number of spawners exceeded escapement goals when forecasts underestimated actual run size, and vice versa, and that forecasts overestimated run size when abundance of returns was low, and vice versa. Our results may change if outcomes of fishing regulations vary in ways not captured by those two relations. Although these relations were empirically based, forecasting accuracy may act as a surrogate for other factors that determine outcome uncertainties.

Second, large stock-to-stock differences in population dynamics and management approaches preclude generalizing our results to other sockeye salmon stocks with confidence, let alone to other species. However, it is more likely that the OU procedure will outperform the base-case management procedure when outcome uncertainty in the "true" population creates a bias between targets and realized outcomes, rather than just imprecision. In addition, it is unlikely that the VM procedure will perform well in other sockeye salmon stocks that show large among-year variability in recruitment (e.g., for cyclic stocks such as Chilko).

Third, within-season forecasts of recruitment of sockeye salmon often replace preseason forecasts fairly early in the fishing season as more accurate estimates of abundance become available, but these within-season forecasts were not explicitly incorporated into our management procedures. However, within-season forecasts were implicit in our relations used to calculate outcome uncertainty in the "true" population because parameters of these relations were estimated from empirical data for years in which management agencies used within-season updates of recruitment. Nevertheless, it is unclear how our results would have changed if we had simulated complex within-season management.

Fourth, although we updated the forecasting submodel with annual recruitment data, we did not incorporate learning into our choice of management targets. Further improvements in performance may be expected with periodic updates of the decision-algorithm submodel (i.e., in a fully closed management feedback loop, sensu Walters 1986), especially if the management procedure and (or) natural variation creates sufficient contrast in spawner abundance to more accurately estimate parameters. Management procedures that include learning when identifying decision algorithms can be evaluated in a simulation model such as the one described here.

Management recommendations

This study shows that assessment methods with more realistic assumptions about the presence and nature of uncertainties may or may not result in improved performance when put in the context of an entire fishery. We therefore recommend that holistic simulations of fisheries be used to evaluate new assessment methods. Although few researchers have applied this approach to salmon, we are not alone in this recommendation. Many previous authors (e.g., de la Mare 1998; Cooke 1999; Punt 2006) suggested that proposed management procedures (including forecasting and decision-algorithm models) should be evaluated in as comprehensive of a simulation model as possible prior to applying them in a fishery. This simulation process was demonstrated for other fisheries such as North Sea plaice (Kell et al. 1999) and the Australian gemfish fishery (Punt and Smith 1999), to name two examples of many. By simulating a fishery over various future scenarios, such evaluations avoid the constraint of assuming that "history will repeat itself", as is implicitly assumed by retrospective analyses of historical data or real-time experiments. Furthermore, as suggested by many of the authors listed above, sensitivity analyses should be used to evaluate management procedures in the face of uncertainties in underlying models that describe system dynamics (McAllister et al. 1999; Punt and Smith 1999). For example, Cooke (1999) recommends considering different assumptions about spatial structure of stocks, trends in productivity over the medium and long terms, and interactions among species, as well as management scenarios with misleading data (i.e., observations and (or) assessments that do not reflect the state of the "true" population). In this way, managers can choose procedures that are relatively robust to structural uncertainty.

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Appendix A. Equations, notations, and parameters used in the simulation model

Table A1 follows.

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Table A1. Description of equations, parameters, and variables used in the simulation model to evaluate management procedures. Paration model in which that equation is used (boxes in Fig. 1). Values are given for parameters in the last two columns. NA represents

Description	Equation
Ricker spawner–recruit relation (used to simulate population dynamics, box 1)	(A1) $R_y = a_r S_y e^{-b_r S_y + \varepsilon_y}$, $\varepsilon_y = \rho\varepsilon_{y-1} + \nu_y$, $\nu \sim N\left(-\frac{\sigma_r^2}{2}, \sigma_r^2\right)$, where the normal deviates are centered around $-\sigma^2/2$ to make the arithmetic mean of the lognormally distributed recruitment multiplier equal to 1
Larkin spawner–recruit relation (to simulate population dynamics, box 1)	(A2) $R_y = a_L S_y^{\prime} e^{(1-b_{L1}S_y - b_{L2}S_{y-1}) + \gamma_y}$, $\gamma \sim N\left(-\frac{\sigma_L^2}{2}, \sigma_L^2\right)$
Mean proportion of recruits-at-age (used to simulate time trends, box 1)	(A3) $\bar{p}_{i,j,t} = a_{i,j} + b_{i,j}t$
Proportion of recruits-at-age calculated from a multivariate logistic distribution (Schnute and Richards 1995) (used to simulate population dynamics, box 1)	(A4) $p_{i,j,t} = \frac{e^{x_{i,j,t}}}{\sum_{j=1}^{J} e^{x_{i,j,t}}}$
Dummy variable used in eq. A4 above	(A5) $x_{i,j,t} = \log_e(\bar{p}_{i,j,t}) + \varpi_i\varepsilon_{i,t} - \frac{1}{J}\sum_{j=1}^{J}[\log_e(\bar{p}_{i,j,t}) + \varpi_i\varepsilon_{i,t}]$, $\varepsilon \sim N(0, 1)$
Proportion of spawners that are effective females (used to simulate population dynamics, box 1)	(A6) $q_y = \text{Beta}(\beta_{q_1}, \beta_{q_2})$
First parameter for eq. A6 above (Morgan and Henrion 1990)	(A7) $\beta_{q_1} = \frac{\mu_q^2 - \mu_q^3 - \sigma_q^2 \cdot \mu_q}{\sigma_q^2}$
Second parameter for eq. A6 above	(A8) $\beta_{q_2} = \frac{\mu_q(1-\mu_q)^2 - \sigma_q^2(1-\mu_q)}{\sigma_q^2}$
Uncertainty in observed spawner abundance (used to simulate observations of abundances, box 2a)	(A9) $S_{O,y} = S_y e^{\kappa_y}$, $\kappa \sim N\left(-\frac{\sigma_\kappa^2}{2}, \sigma_\kappa^2\right)$

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meters and variables are defined only at their first occurrence. The description in the first column includes the component of the simula-
parameters that were not used for that stock; blank cells in the last two columns correspond to variables.

Parameter or variable	Definition	Values	
		Togiak	Chilko
y	Brood year		
r	Index designating parameters of the Ricker stock–recruitment relation		
R	Abundance of recruits		
S	Abundance of spawners		
a_r, b_r	Parameters of the Ricker spawner–recruit relation	5.55, 3.75×10^{-6}	NA
ϕ, ν	Stochastic terms		
ρ	Autocorrelation coefficient for the residual errors	0.25	NA
σ_r	Standard deviation in residuals	0.51	NA
L	Index designating parameters of the Larkin stock–recruitment relation		
S'	The number of effective female spawners ($S\times q$, see eq. A6)		
a_L, b_{L1}, b_{L1}	Parameters	NA	19.84, 2.25×10^{-6}, 2.33×10^{-6}
γ	Stochastic term		
σ_L	Standard deviation in residuals	NA	0.63
i	Number winters spent in freshwater		
j	Number of winters spent in the ocean		
$\bar{p}_{i,j}$	Mean proportion of recruits that spend i winters in freshwater and j in the ocean		
t	Simulated calendar year at return (t = 1–50)		
$a_{i,j}$, $b_{i,j}$	Y intercept and slope of relation between year t and $\bar{p}_{i,j}$	$a_{1,2}$ = 0.36, $b_{1,2}$ = -3.8×10^{-5}, $a_{1,3}$ = 0.64, $b_{1,3}$ = 3.7×10^{-5}, $a_{2,2}$ = 0.51, $b_{2,2}$ = -3.5×10^{-5}	$a_{1,1}$ = 0.016, $b_{1,1}$ = -1.7×10^{-4}, $a_{1,2}$ = 0.99, $b_{1,2}$ = -2.0×10^{-3}
p	Proportion of recruits-at-age including multivariate logistic error		
j_i	Number of winters spent in the ocean for i winters spent in freshwater		
J_i	Maximum number of winters spent in the ocean for i winters spent in freshwater	4 for i = 1 and 3 for i = 2	3 for i = 1
$x_{i,j}$	Defined by eq. A5 below		
ω_i	Standard deviation in the multivariate logistic distribution of proportions-at-age	0.4 for i = 1 and 0.6 for i = 2	0.9 for i = 1
ε	Stochastic term		
q	Proportion of spawners that are effective females		
β_{q1}, β_{q2}	Described in eqs. A7 and A8 below		
μ_q	Mean proportion of spawners that are effective females	NA	0.53
σ_q	Standard deviation in proportion of spawners that are effective females	NA	0.092
S_O	Observed abundance of spawners		
κ	Stochastic term		
σ_κ	Standard deviation in observation error of spawner abundances	0.02	0.02

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Table A1 (*concluded*).

Description		Equation
Sibling model (used to forecast age-specific recruitment, box 2*b*)	(A10)	$\log_e(R_{i,t,t}) = a'_{i,t,t} + b'_{i,t}\log_e(R_{i,t-1,t-1}) + \omega_{i,t,t}$, $\omega_{i,t} \sim N(0, {\sigma_{\omega_{i,t}}}^2)$
Sibling model, system equation (used along with eq. A10 in the Kalman filter version to forecast age-specific recruitment, box 2*b*)	(A11)	$a'_{i,t,t} = a'_{i,t,t-1} + \xi_{i,t,t}$, $\xi_{i,t} \sim N(0, {\sigma_{\xi_{i,t}}}^2)$
Target harvest rule ignoring outcome uncertainty (generated from decision-algorithm submodel, box 3, and used to apply target harvest rates, box 4)	(A12)	$h'_t = c\{1 - \exp[d(m - R'_t)]\}$
Same as eq. A12 except that this target harvest rule accounts for outcome uncertainty	(A13)	$h'_t = c^*\{1 - \exp[d^*(m^* - R'_t)]\}$
Deviation of realized escapement from target for Togiak only (used to simulate realized escapements in the decision-algorithm submodel, box 3, and when applying target escapements, box 4)	(A14)	$\log_e\left(\frac{E_t}{E'_t}\right) = \lambda_0 + \lambda_1 F_t + \delta_t$, $\delta \sim N(0, \sigma_\delta^2)$
Outcome uncertainty in harvest rates for Chilko only (used to simulate realized harvest rates in the decision-algorithm submodel, box 3, and when applying target harvest rates, box 4)	(A15)	$h_t = \text{Beta}(\beta_{h_{t,1}}, \beta_{h_{t,2}})$
First parameter for eq. A15 above (Morgan and Henrion 1990)	(A16)	$\beta_{h_{t,1}} = \frac{h'^2_t - h'^3_t - \sigma^2_h \cdot h'_t}{\sigma^2_h}$
Second parameter for eq. A15 above	(A17)	$\beta_{h_{t,2}} = \frac{h'_t(1 - h'_t)^2 - \sigma^2_h(1 - h'_t)}{\sigma^2_h}$
Escapement after harvest (used to simulate realized escapements in the decision-algorithm submodel, box 3, and when applying target escapements, box 4)	(A18)	$E_t = R_t(1 - h_t)$

Appendix B. Separating sources of uncertainty

Separating observation error from natural variability

Historical recruitment data used to estimate parameters of eqs. A1 and A2 (Appendix A, Table A1) contain both natural process variation and observation error; hence, recruitment calculated from these equations contained both ν or γ (eqs. A1 and A2, respectively) and κ (eq. A9). To separately estimate their respective contributions to variability, we first set the standard deviation in observation error of the natural logarithm of spawner abundance, σ_κ, to 0.02, the mean estimate for observation error in visual surveys of escapement for Togiak in 2004 and 2005 (T. Baker, Alaska Department of Fish and Game, 333 Raspberry Road, Anchorage, AK 99518-159, USA, personal communication). For that stock, the precision of the sampling method was reported as coefficients of variation (CV = standard deviation/mean) and were converted to variance, σ^2 (for the error term in eq. A9), using the well-known relationship

$$\text{(B1)} \qquad \sigma^2 = \log_e(\text{CV}^2 + 1)$$

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Parameter or variable	Definition	Values	
		Togiak	Chilko
$R_{i,j}$	Abundance of recruits that spent i winters in freshwater and j winters in the ocean		
$a^*_{i,j,t}$	Time-varying parameter	Estimated annually using the Kalman filter	
$b^*_{i,j}$	Constant parameter	Estimated for each stock and age class separately	
ω	Stochastic term		
$\sigma_{\omega,i,j}$	Standard deviation of "observation error"	Estimated for each stock and age class separately	
ξ	Stochastic term		
$\sigma_{\xi,i,j}$	Standard deviation of "process error"	Estimated for each stock and age class separately	
h'	Target harvest rate		
R'	Forecast of recruitment		
c	Maximum harvest rate (proportion) at high R' for the target harvest rule that ignores outcome uncertainty	NA	0.93
d	Shape parameter for that harvest rule	NA	1.20×10^{-6}
m	Protection level (spawner abundance below which harvest rates are zero for that harvest rule)	NA	1
c^*, d^*, m^*	Same as c, d, and m in eq. A12, except this model accounts for outcome uncertainty	NA	0.93, 9.07×10^{-6}, 1
F	Forecasting error ($\log_e(R'/R)$)		
δ	Stochastic term		
E	Realized escapement		
E'	Target escapement that ignores outcome uncertainty	179 176	NA
	Target escapement that accounts for outcome uncertainty	132 660	NA
λ_0, λ_1	Parameters	0.26, –0.40	NA
σ_δ	Standard deviation in residuals	0.19	NA
h	Realized harvest rate		
$\beta_{h_0,t}$, $\beta_{h_1,t}$	Described in eqs. A16 and A17 below		
σ_h	Standard deviation in harvest rates	NA	0.18

The standard deviation ($\sqrt{\sigma_\omega^2}$) in observed error for Togiak corresponded to a relatively low level of observation error for Chilko, as shown by mark–recapture studies on 10 Fraser River stocks, where annual estimates of σ_ω ranged from 0.02 to 0.19 between 2001 and 2004 (T. Whitehouse, Fisheries and Oceans Canada, B.C. Interior Area and Science Branch, 985 McGill Place, Kamloops, BC V2C 6X6, Canada, personal communication). In a simple simulation model, we then estimated the standard deviations of the natural process variation, σ_t and σ_L (for eqs. A1 and A2 for Togiak and Chilko, respectively), which in combination with the above observation errors in the simulation resulted in standard deviations in total recruitment equal to those observed historically (σ_ω). Note that those variances were not additive (i.e., $\sigma_\omega^2 \neq \sigma_t^2 + \sigma_\omega^2$ for Togiak and $\sigma_\omega^2 \neq \sigma_L^2 + \sigma_\omega^2$ for Chilko), indicating that the sources of variability were not independent. For both of our study populations, we found that the total combined uncertainty (σ) was dominated by large, natural variability in recruitment. Specifically, the standard deviation in natural process variation was equal to 99% of the standard deviation in total combined recruitment for both stocks (i.e., $\sigma_t = 0.99\sigma_\omega = 0.51$ in eq. A1 for Togiak, and $\sigma_L = 0.99\sigma_\omega = 0.63$ in eq. A2 for Chilko). In a sensitivity analysis, we increased the magnitude of observa-

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tion errors to $\sigma_x = 0.19$, which corresponded to standard deviations in natural process variation that were equal to 94% of the standard deviation in total observed recruitment for Togiak (i.e., $\sigma_r = 0.94\sigma_y = 0.48$) and 90% for Chilko (i.e., $\sigma_L = 0.90\sigma_y = 0.57$).

Separating observation error from outcome uncertainty

We separated observation error (σ_x, eq. A9) from outcome uncertainty (σ_b, eq. A14, for Togiak and σ_b, eqs. A15–A17, for Chilko) using the same method as described above. For Togiak, the standard deviation in outcome uncertainty not included in observation error was 94% of the standard deviation in total observed outcome uncertainty that included observation error (resulting in $\sigma_b = 0.19$), and that proportion was 99% for Chilko (resulting in $\sigma_b = 0.18$). When we increased observation errors in a sensitivity analysis ($\sigma_x = 0.19$ instead of 0.02), these values (σ_r and σ_b) were 70% and 88% for Togiak and Chilko, respectively (resulting in $\sigma_r = 0.14$ and $\sigma_b = 0.16$, respectively).

Appendix C. Decision-algorithm submodel

The decision-algorithm submodel used the following five simplifications of the overall simulation that evaluated management options.

(1) Assumptions about recruitment

For Togiak, the decision-algorithm submodel assumed that all recruits were age 5 years at maturity, because from 1956 through 2001, on average, 67% of recruits were age 5. For Chilko, the decision-algorithm submodel assumed that adults returned at ages 4 and 5 because ecological interactions between fish that return in subsequent years, in part due to differential age-at-maturity, are an important component of cyclic population dynamics of Chilko Lake sockeye salmon (Ricker 1997). The proportion of recruits that were age 4 in year t, $p_{4,t}$, was calculated from the proportion of spawners that were age 4 in the parent generation, $p'_{4,t}$ (Walters and Woodey 1992), using the following logistic model:

$$(C1) \qquad p_{4,t} = (1 + f_0 f_1^{(p'_{4,t})})^{-1} + \tau_t$$

and

$$\tau \sim N(0, \sigma_\tau^2)$$

where f_0 and f_1 are parameters and τ represents normally distributed random variation with variance σ_τ^2. The dependent variable, $p_{4,t}$, was always between zero and one.

(2) Assumptions about time trends in age-at-maturity

In contrast to the overall simulation model, we did not incorporate long-term trends in age-at-maturity in the decision-algorithm submodel.

(3) Assumptions about forecasts of recruitment

In the decision-algorithm submodel, we did not explicitly simulate forecasts of recruitment. Instead, for Togiak, we simulated only errors in forecasts, and for Chilko, we did not simulate forecasts or errors in forecasts because historical data showed that outcomes of fishing regulations were independent of those factors. Specifically, for Togiak, we simulated a stock assessment procedure that took into account forecasting error from an empirically based relation with realized recruitment. Our analyses of historical data for that stock show that forecasts of recruitment tend to underestimate realized recruitment when recruitment is large and overestimate realized recruitment when recruitment is small. Therefore, we calculated forecasting error (the natural logarithm of the ratio of forecasts of recruitment, R'_t, to realized recruitment, R_t) from the natural logarithm of realized recruitment:

$$(C2) \qquad \log_e\left(\frac{R'_t}{R_t}\right) = \eta_0 + \eta_1 \log_e(R_t) + \alpha_t$$

and

$$\alpha \sim N(0, \sigma_\alpha^2)$$

where η_0 and η_1 are parameters (equal to 13.5 and –1.02, respectively), and α represents normally distributed variability in forecasting errors with variance, σ_α^2 (= 0.35). We used log-transformed abundances because of empirical and theoretical support for lognormally distributed variation in recruitment (Peterman 1981; Hilborn and Walters 1992).

(4) Assumptions about observation errors

In the decision-algorithm submodel, observation errors were not separated from outcome uncertainty and natural variability (as described for the overall model that we used to evaluate management options, see Appendix B) because estimates of observed abundance were not explicitly simulated.

(5) Number of Monte Carlo trials

The decision-algorithm submodel was repeated over 1000 iterations instead of the 500 used for the overall simulation model because the decision-algorithm submodel did not included time trends in age-at-maturity and therefore was not iterated over years. Therefore, a larger sample of Monte Carlo trials was required to stabilize output metrics in this decision-algorithm submodel.

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An improved method for predicting the accuracy of genetic stock identification

Eric C. Anderson, Robin S. Waples, and Steven T. Kalinowski

Abstract: Estimating the accuracy of genetic stock identification (GSI) that can be expected given a previously collected baseline requires simulation. The conventional method involves repeatedly simulating mixtures by resampling from the baseline, simulating new baselines by resampling from the baseline, and analyzing the simulated mixtures with the simulated baselines. We show that this overestimates the predicted accuracy of GSI. The bias is profound for closely related populations and increases as more genetic data (loci and (or) alleles) are added to the analysis. We develop a new method based on leave-one-out cross validation and show that it yields essentially unbiased estimates of GSI accuracy. Applying both our method and the conventional method to a coastwide baseline of 166 Chinook salmon (*Oncorhynchus tshawytscha*) populations shows that the conventional method provides severely biased predictions of accuracy for some individual populations. The bias for reporting units (aggregations of closely related populations) is moderate, but still present.

Résumé : L'estimation de la précision de l'identification du stock génétique (« GSI ») qu'on peut espérer, étant donné une banque de données de base récoltée antérieurement, nécessite des simulations. La méthode courante comprend des simulations répétées de mélanges par ré-échantillonnage de la banque de données de base, des simulations de nouvelles banques de données de base en ré-échantillonnant la banque de données et l'analyse des mélanges ainsi simulés à l'aide des banques de données de base simulées. Nous montrons que cette méthode surestime la précision prédite de GSI. L'erreur est importante dans les populations fortement apparentées et elle augmente à mesure que de nouvelles données génétiques (locus et (ou) allèles) sont ajoutées à l'analyse. Nous mettons au point une nouvelle méthode basée sur une validation croisée de type « leave-one-out » (avec retrait d'un élément) et nous montrons qu'elle produit essentiellement des estimations non erronées de la précision de GSI. L'application de notre méthode et de la méthode courante à une banque de données de base provenant de 166 populations de saumons chinook (*Oncorhynchus tshawytscha*) réparties sur toute la côte montre que la méthode courante fournit des prédictions de la précision qui sont grandement faussées pour certaines populations individuelles. L'erreur dans le cas des unités d'évaluation (des rassemblements de populations fortement apparentées) est peu importante, mais réelle.

[Traduit par la Rédaction]

Introduction

Genetic data have been used to estimate the stock composition of mixed-stock fisheries for over two and a half decades (e.g., Grant et al. 1980; Milner et al. 1981). The basic methodology for this is straightforward: fish from the mixed fishery are genotyped, as are fish in "baseline" samples, which are taken separately from the populations that might contribute to the mixture. Mixture proportions in the fishery are then estimated using conditional maximum likelihood (Milner et al. 1981; Fournier et al. 1984; Millar 1987), unconditional maximum likelihood (Smouse et al. 1990), or Bayesian (Pella and Masuda 2001, 2006) methods that relate the genotypes in the mixture to the expected genotype frequencies in the baseline populations. Most applications of this sort of genetic stock identification (GSI) have involved Pacific salmon, but the basic methodology has now been applied to a wide range of other species as well (Waldman et al. 1997; McParland et al. 1999; Koljonen et al. 2005).

Initially, allozyme polymorphisms used for GSI provided sufficient resolution to address many Pacific salmon management concerns; however, other types of genetic data are now becoming increasingly abundant and inexpensive. Highly polymorphic microsatellite loci have been shown to provide considerable power for GSI (Kalinowski 2004; Winans et al. 2004). Declining costs of genotyping and the recent completion of a cross-laboratory, standardized, microsatellite DNA baseline for over 150 populations throughout the range of Chinook salmon (Seeb et al. 2007) have led to microsatellites replacing allozymes for many GSI applications. In addition, numerous single nucleotide polymorphisms (SNPs) are being discovered in Pacific salmon, and these may soon become useful, low-cost, high-throughput genetic markers for GSI (Smith et al. 2005*a*). At the same

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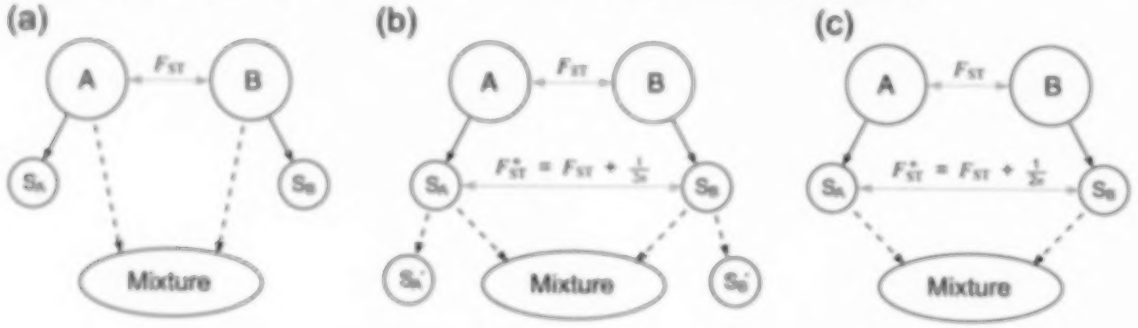
Fig. 1. Diagrams representing different ways of simulating mixtures for the assessment of genetic stock identification (GSI) accuracy with two populations (A and B) in the baseline. Both baseline samples, S_A and S_B, are assumed to be of size n individuals. Broken arrows represent the simulation of genotypes. (*a*) Individuals in the mixture are drawn directly from parametric population allele frequencies, as are the baseline samples used to analyze the mixture. The magnitude of difference in parametric allele frequencies between A and B is indicated by F_{ST}. Pursuing this method is typically impossible because the allele frequencies in A and B are never known without error. (*b*) The conventional method in use today: parametric bootstrap with baseline resampling (PB-R). The baseline sample provides estimates of allele frequencies, and these estimates are used to generate simulated individuals for the mixture and for a new "resampled" baseline. On average, the magnitude of allele frequency difference between the populations, estimated from the baseline (F'_{ST}), will be larger than between the true population allele frequencies; $F'_{ST} = F_{ST} + \frac{1}{2n}$. As a result, individuals in the mixture will be genetically more divergent than is really the case, leading to overly optimistic estimates of power. (*c*) As in *b*, but without baseline resampling (PB-NR).



time, there is a growing need for these technologies in fisheries management: attempts are being made not only to apply GSI to mixtures of evermore genetically similar stocks, but also to identify the origin of individual fish in mixed fisheries rather than just the aggregate proportion of fish from different populations. These management tasks require highly informative genetic data.

This confluence of management needs and data availability makes it imperative that fisheries geneticists have at their disposal a reliable method for assessing the expected accuracy of GSI with a given baseline data set. Developing such a method is the main purpose of this paper. GSI is most accurate when there is a high degree of genetic differentiation between populations, when the baseline samples for each population are large, and when large numbers of loci are genotyped (Wood et al. 1987; Kalinowski 2004), but there is no simple formula available to calculate how accurate GSI is expected to be; therefore computer simulation must be used. However, as we explain below, the simulation methods currently in use to do this are flawed in a way that leads them to consistently overestimate the expected accuracy of GSI.

If parametric allele frequencies in source populations could be known without error, then estimating the accuracy of GSI via computer simulation would be straightforward (Fig. 1*a*). Baseline and mixture samples could be simulated from the allele frequencies in the source populations, and these data could be used to estimate mixture proportions. The mean squared error (or some other summary statistic) for a large number of these estimates would provide an estimate of how accurate GSI is likely to be. In reality, parametric allele frequencies of source populations are never known exactly, so an alternative simulation procedure must be used. The standard approach in such situations is to assume that the parametric allele frequencies in the source populations are equal to the observed allele frequencies in the baseline samples taken from the source populations (Fig. 1*b*). Baseline and mixture genotypes are then simulated from these allele frequencies in the same manner as they are when parametric allele frequencies are known (Fournier et al. 1984; Beacham et al. 2006). The only difference is that the allele frequencies in the baseline samples are used as if they were the parametric allele frequencies.

This approach for estimating the accuracy of GSI has been in use for a long time in a variety of computer packages (including some of our own) and produces reasonable estimates of the accuracy of GSI in many circumstances; however, as we show below, GSI is generally less accurate than these conventional simulations predict it will be, and the discrepancy is most severe for the most challenging applications of GSI. The crux of the problem is that conventional simulation does not properly account for sampling error in baseline allele frequencies, and this error induces a spurious correlation between the baselines and the individuals simulated by resampling from the baseline. Sampling error makes the allele frequency divergence observed between samples larger, on average, than the true differences between the populations. Using these observed sample allele frequencies to naively drive simulations of GSI will lead one to exaggerate the accuracy of GSI. The magnitude of this problem can be understood heuristically in terms of F_{ST}, a standardized measure of genetic differentiation between populations that is familiar to most population geneticists. If sampling error is not accounted for, F_{ST} for two samples from a pair of populations will be inflated by a term of approximate magnitude $1/(2n)$ (Wright 1978; Chakraborty and Leimar 1987), where n is the number of individuals sampled. The estimated value of F_{ST}, F'_{ST}, between two source populations will then be equal to the true value plus $1/(2n)$. Because the accuracy of GSI increases with F_{ST}, an inflated F'_{ST} will lead to overly optimistic assessments of how accurate GSI is likely to be.

A few numerical examples illustrate the potential importance of this type of bias. Assume that the parametric F_{ST}

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between source populations A and B is 0.02 and that the baseline samples, S_A and S_B, for these populations each include $n = 50$ individuals. The expected magnitude of inflation of $F_{ST}^{\wedge}$ due to sampling error is then $1/(2n) = 0.01$. In this case, the degree of genetic differentiation that characterizes individuals simulated for the mixture is $F_{ST}^{\wedge} = 0.02$ (parametric) + 0.01 (baseline sampling) = 0.03, which is inflated by 50% compared with the true F_{ST} for the populations. In the extreme, if A and B are really parts of the same panmictic population, there is no basis whatsoever for resolving a "mixture" of A and B individuals. However, a mixture analysis conducted as in Fig. 1*b* would suggest that some ability to resolve this mixture does exist. Furthermore, as more and more loci are added to the analysis, the apparent power to resolve this spurious mixture will increase. If, on the other hand, allele frequency differences between populations actually are much larger (e.g., $F_{ST} = 0.1$), the mixture analysis will have high intrinsic power, and the relatively small inflation of $F_{ST}^{\wedge}$ due to baseline sampling will have comparatively little effect on estimated power.

In this paper, we propose a new method based on a leave-one-out procedure for assessing GSI accuracy that is easy to implement and that reduces the bias to a negligible amount in most cases. Leave-one-out cross validation is not new to genetic classification problems (Spielman and Smouse 1976; Piry et al. 2004). However, the utility of the leave-one-out procedure has not been fully appreciated in the context of GSI, perhaps because its application to GSI is not as straightforward or obvious as its application to genetic assignment tests. We illustrate the utility of the new method and evaluate the magnitude of the bias in the conventional method using simulated data. Finally, we assess GSI accuracy in a large microsatellite baseline using both the conventional biased method and our new method to demonstrate the practical implications of the bias.

Methods

Once a baseline sample, which includes, say, M populations, has been collected, it is natural to inquire how accurately that baseline can be used to estimate the mixed-stock fishery proportions, $\boldsymbol{\pi} = (\pi_1, \ldots, \pi_M)$, using a fishery sample of size N fish that might be collected in the future. For this exercise, it is typical to assume some value $\boldsymbol{\pi}^*$ of the mixing proportions and perform simulations to see how closely the maximum likelihood estimates, $\widehat{\boldsymbol{\pi}}$, correspond to $\boldsymbol{\pi}^*$. The conventional method to do this, as implemented in SPAM (Alaska Department of Fish and Game 2000) and GMA (Kalinowski 2003), involves the following steps for each simulated data set. (1) The number of fish in the mixture from each of the M populations is drawn from a multinomial distribution of N trials with cell probabilities $\boldsymbol{\pi}^*$. (2) A genotype $\boldsymbol{G}$ for each fish in the mixture from population i is simulated by sampling, with replacement, from population i's allele frequencies, $\widehat{\boldsymbol{\theta}}_i$, as estimated from the observed baseline. (3) A new baseline sample of the same size as the observed baseline is created by sampling alleles, with replacement, from the observed baseline. A genotype for each fish in the simulated baseline from population i is simulated by sampling with replacement from $\widehat{\boldsymbol{\theta}}_i$. (4) The simulated baseline is applied, using the standard conditional-likelihood GSI framework (Fournier et al. 1984; Millar 1987), to find the conditional MLE, $\widehat{\boldsymbol{\pi}}$, from the simulated mixture.

The conditional likelihood function commonly in use today is an integrated likelihood that assumes a unit-information Dirichlet prior on allele frequencies (Rannala and Mountain 1997). In such a case, genotypes can be simulated from their posterior predictive distribution (a compound Dirichlet–multinomial distribution) rather than by simply sampling alleles with replacement from the baselines. There seems to be little practical difference between these two approaches.

Although the unconditional likelihood framework for GSI (Smouse et al. 1990) and Bayesian methods (Pella and Masuda 2001, 2006) may be preferable to a conditional likelihood analysis, the conditional likelihood framework is usually used for analyzing the simulated data sets because it is customary to analyze many simulated data sets, and maximizing the conditional likelihood requires far less computer time than maximizing the unconditional likelihood or computing the Bayesian posterior distribution.

We call the above method the parametric bootstrap method with baseline resampling (PB-R). A second method, which is available as an option in SPAM, is the parametric bootstrap with no baseline resampling (PB-NR). PB-NR is identical to PB-R except that step 3 is omitted and the simulated mixture is analyzed with the original baseline sample. Our simulations (see below) show that both PB-R and PB-NR provide upwardly biased predictions of GSI accuracy.

We introduce a slight modification to the steps above to provide an essentially unbiased method for predicting GSI accuracy. It proceeds as follows: step 1 is unmodified; step 2 is slightly modified, the two gene copies at a locus in a single individual are drawn without replacement (but they are both replaced before the next individual is simulated); step 3 is omitted (it is not necessary to simulate a new baseline sample); and in step 4, a leave-one-out procedure is used when computing $P(\boldsymbol{G}|\widehat{\boldsymbol{\theta}}_i)$, the probability of the simulated fish's genotype $\boldsymbol{G}$ given the baseline allele frequencies. This leave-one-out procedure takes the following form: if i is not the population from which the individual was simulated, then $P(\boldsymbol{G}|\widehat{\boldsymbol{\theta}}_i)$ is computed as before. If, however, i is the population from which the individual was simulated, then $P(\boldsymbol{G}|\widehat{\boldsymbol{\theta}}_i^{(-)})$ is used instead of $P(\boldsymbol{G}|\widehat{\boldsymbol{\theta}}_i)$. $\widehat{\boldsymbol{\theta}}_i^{(-)}$ is just the MLE of $\boldsymbol{\theta}_i$ computed after subtracting the gene copies carried in $\boldsymbol{G}$ from the genes found in the baseline from population i.

We refer to this new method as the cross-validation method over gene copies (CV-GC). We explore two other methods as well: cross validation over single locus genotypes (CV-SL) and over multilocus genotypes (CV-ML). CV-SL is identical to CV-GC except that in step 2, each single-locus genotype is simulated by randomly sampling, with equal probability, from among the single-locus genotypes carried by members of the appropriate population in the baseline. CV-ML is identical to CV-GC except that in step 2 each individual's full, multilocus genotype is simulated by randomly sampling, with equal probability, from among the multilocus genotypes carried by members of the appropriate population in the baseline.

Some notation will be useful for the following section: we

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use $\bar{T}_j$ to denote the scaled likelihood vector of the jth individual in a mixture. If the jth individual has genotype G, this vector is obtained by starting with a vector the elements of which are all $P(G|\theta_i)$ and scaling them to sum to one. It shall be understood that when using any of the leave-one-out, cross-validation methods, one of the elements of $\bar{T}_j$ will have been computed using $P(G|\hat{\theta}_i^{(-j)})$. It can be shown by the factorization theorem (Casella and Berger 1990, p. 250) that the set of all $\bar{T}_j$s is the sufficient statistic for π, and a mathematical justification for our cross-validation methods may be described in terms of the distribution of simulated values of this sufficient statistic; however, for brevity, we omit those details. The simulation results provide enough evidence of the utility of our new methods.

Simulation experiments

We perform three separate experiments using purely simulated data. In the first two, the structured coalescent model (Hudson 1990) implemented in *makesamples* (Hudson 2002) is used to simulate genotypes of individuals from different populations in both the baseline and the mixture samples. Each locus was simulated using a separate, independent realization of the coalescent process on a nonrecombining DNA segment that we will refer to as a "chromosome". This creates loci that are independently segregating. An island model of migration was used to effect a given degree of divergence between the populations. Under this model, populations that exchange many migrants each year are not very highly diverged, and populations that exchange few migrants each year will be more highly diverged. In *makesamples*, the migration rates are parameterized by a migration matrix $[4N_e m_{i,j}]$, where $m_{i,j}$ is the fraction of population i that is composed of migrants from population j each generation and N_e is the effective size of each population. N_e was assumed to be equal among all populations and constant in time.

For example, to simulate 10 microsatellite loci in 200 individuals (400 chromosomes) from each of three populations in a symmetric island model with $4N_e m_{i,j} = 5$, we would first use the *makesamples* command line "ms 1200 10 -t 8.0 -I 3 400 400 400 10". The above produces data using the infinite sites model of mutation with rate controlled by the parameter $4N_e\mu = 8.0$, where μ is the rate of neutral mutation each generation. Each mutation in the infinite-sites model corresponds to a mutation at a single, unique nucleotide on the chromosome. For each chromosome in the simulation, *makesamples* returns an ordered string of 1s and 0s, which denote whether the chromosome did or did not, respectively, inherit the mutated form of the nucleotide. We convert those ordered arrays of infinite-sites mutations to microsatellite alleles following a stepwise mutation model with occasional multistep mutations in the following manner: the ancestral microsatellite allele length is set to a large value and each mutant nucleotide in the locus is randomly assigned to be an allele length increase or decrease with equal probability. The magnitude of the length change associated with each mutant nucleotide with probability of 0.85 was chosen to be 1 and with probability of 0.15 was $2 + W$, where W is drawn from a Poisson distribution with a mean of 3. Our mutation parameters yielded simulated loci with an average of 15 alleles. The simulation of microsatellite alleles from infinite sites mutations was done using the program *ms2geno* available from E. Anderson upon request.

For each simulation replicate, the genotypes of a large number of individuals from each population were simulated. Some of those individuals were randomly assigned to be members of the baseline samples, and other, separate individuals were chosen to be members of the mixture samples. We simulated different numbers of individuals under varying parameter values in the two coalescent simulation experiments. Specifics are described below. The number of individuals in the baseline sample from population p is denoted by n_p, the number of individuals from population p in the mixture is denoted by N_p, and the size of the whole mixture sample is denoted by N.

All the simulation output was analyzed using the program *gsi_sim* written by Eric Anderson. The programs GMA, SPAM, and cBayes (Neaves et al. 2005) are not amenable to large-scale simulation studies because they are available only for the Microsoft Windows platform and cannot be easily scripted in a Unix-like environment. We verified the results of our large simulations on small test cases analyzed by GMA and SPAM. All of the analyses, and the parametric bootstrapping of mixtures, were done using the likelihood model proposed by Rannala and Mountain (1997) with the unit-information prior. Nearly identical results were obtained using the uniform prior (result not shown).

Our first experiment investigates the simplest population scenario possible: a mixture formed from two populations, indexed 1 and 2, simulated with a migration rate $4N_e m_{1,2} = 4N_e m_{2,1} \in \{\text{PANMIX}, 1000, 250, 100, 25, 10\}$ between them. We use "PANMIX" to denote the case in which the populations are entirely panmictic. It is sometimes easier to think of these migration rates in terms of the expected F_{ST} values that they will produce between the populations. Under an infinite alleles model, the expected F_{ST} values would be approximately 0, 0.001, 0.004, 0.01, 0.04, and 0.09, respectively. Mutation rate was $4N_e\mu = 4.5$ (for the PANMIX case, $4N_e\mu$ was set to 8.0 to ensure roughly the same number of alleles in all cases). Baseline sample sizes from each of the two populations were $n_1 = n_2 = 144$, and each individual had genotypes at $L = 13$ loci. These sample sizes and L are identical to those throughout the baseline gathered by the Pacific Salmon Commission (PSC) Genetic Analysis of Pacific Salmon (GAPS) Consortium (Seeb et al. 2007). We compared the true distributions of $\bar{T}_j$ and $\hat{\pi}$ with the distributions obtained by simulating new individuals using resampling from the baseline allele frequencies. The true distributions, which we refer to as "true", were obtained by computing $\bar{T}_j$ and estimating $\hat{\pi}$ from mixtures containing individuals that were distinct from the individuals in the baselines, but which were simulated on the same coalescent trees. The distributions of $\bar{T}_j$ and $\hat{\pi}$ for mixtures formed by resampling from the baseline allele frequencies were obtained using the PB-NR, PB-R, CV-ML, CV-SL, and CV-GC methods described above. Mixtures of $N = 400$ were simulated. For assessing the distributions of $\bar{T}_j$, $N_1 = 400$ and $N_2 = 0$ and we investigated the distribution of the mean over all fish j of $\bar{T}_{j,1}$. The distribution of $\hat{\pi}$ was assessed in the simulation with $N_1 = 400$ and another with $N_1 = 250$. Note

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Table 1. Comparison of the CV-GC and PB-R methods using the GAPS microsatellite baseline (version 2.1 of the baseline, unpublished data, obtained in 2006 from M. Banks, Coastal Oregon Marine Experiment Station, Hatfield Marine Science Center, Department of Fisheries and Wildlife, Oregon State University, Newport, OR 97365) and mixing proportions from the 2006 Monterey Bay recreational fishery.

			Mean π_i		$\sqrt{\text{MSE of } \pi_i}$		Fraction of individual assignments correct	
Region or reporting unit	Population	True π	CV-GC	PB-R	CV-GC	PB-R	CV-GC	PB-R
Central Valley Fall	Feather Hatchery Fall	0.3298	0.3374	0.3441	0.0337	0.0377	0.504	0.746
Central Valley Spring	Feather Hatchery Spring	0.2731	0.2704	0.2742	0.0292	0.0303	0.596	0.773
Central Valley Fall	Battle Creek	0.2069	0.2062	0.2107	0.0265	0.0272	0.513	0.739
Central Valley Fall	Stanislaus River	0.0570	0.0526	0.0465	0.0168	0.0176	0.151	0.498
Central Valley Fall	Butte Creek Fall	0.0489	0.0467	0.0377	0.0156	0.0169	0.145	0.462
Central Valley Winter	Sacramento Hatchery	0.0321	0.0317	0.0320	0.0086	0.0088	0.999	1.000
Central Valley Spring	Butte Creek Spring	0.0150	0.0147	0.0148	0.0063	0.0062	0.905	0.947
Central Valley Spring	Mill Creek Spring	0.0083	0.0088	0.0086	0.0053	0.0049	0.552	0.748
Rogue River	Applegate Creek	0.0077	0.0063	0.0072	0.0038	0.0040	0.732	0.854
California Coast	Eel River	0.0039	0.0036	0.0037	0.0024	0.0024	0.896	0.952
California Coast	Russian River	0.0030	0.0032	0.0031	0.0024	0.0024	0.909	0.945
Klamath River	Trinity Hatchery Spring	0.0028	0.0026	0.0027	0.0023	0.0023	0.646	0.788
Northern California – southern Oregon Coast	Chetco River	0.0026	0.0025	0.0026	0.0020	0.0021	0.900	0.942
Klamath River	Klamath River Fall	0.0024	0.0023	0.0024	0.0021	0.0021	0.733	0.845
Klamath River	Trinity Hatchery Fall	0.0020	0.0023	0.0022	0.0024	0.0022	0.636	0.766
Central Valley Spring	Deer Creek Spring	0.0018	0.0019	0.0017	0.0023	0.0018	0.303	0.610
Upper Columbia River	Hanford Reach	0.0014	0.0012	0.0016	0.0019	0.0021	0.453	0.667
Rogue River	Cole Rivers Hatchery	0.0005	0.0012	0.0008	0.0020	0.0015	0.587	0.750
Mid-Oregon Coast	Umpqua Hatchery	0.0005	0.0009	0.0007	0.0017	0.0015	0.510	0.723
Mid-Oregon Coast	Coquille River	0.0005	0.0003	0.0004	0.0007	0.0009	0.557	0.736

Note: True π refers to the value of π used to generate the simulated mixtures. CV-GC, cross-validation method over gene copies; PB-R, parametric bootstrap with baseline resampling. Summary statistics for $\hat{\pi}$, and the fractions of correct individual assignments were calculated from 2500 simulated mixed-fishery samples.

that in this case, the proportion of fish from the two populations is fixed in the sample itself. Thus the variance of $\hat{\pi}$ does not include the variance due to finite sampling of fish from the mixture. For each value of N_1, 1000 simulated baselines and mixtures were used. For analyses involving resampling, a single mixture was simulated and analyzed per baseline. Thus, each distribution is estimated using 1000 realizations of the variable.

In the second experiment, we simulated five populations in a symmetric island model with migration rates $4N_em_{i,j} \in \{\text{PANMIX}, 62.5, 25, 6.25, 2.5\}$ for all $i \neq j$. In a single coalescent simulation for each migration rate, we generated enough individuals to produce 25 replicate baselines containing 144 individuals from each of the five populations and 250 mixture samples of size $N = 400$. The true mixing proportions $\boldsymbol{\pi}$ for populations 1–5 were 0.5, 0.25, 0.125, 0.0625, and 0.0625, respectively. We analyzed each mixture sample using the 25 replicate baselines. Additionally, using both the CV-GC and the PB-R methods, for each of the 25 baselines, we created and analyzed 250 mixtures by resampling genes from the baselines. For each of the 25 baselines, the average value of $\hat{\pi}$ over the 250 mixtures was recorded. The results are presented in terms of the mean and standard deviation of this mean $\hat{\pi}$ over the 25 baselines considered across the five different populations and the five different migration rates.

The third and final simulation was designed to determine whether the bias of the PB-R method is a complication of using loci with large numbers of alleles, some of which are at very low frequency, or whether the bias is evident even when the loci used have few alleles, all of them at intermediate frequency. Kalinowski (2004) found that the total number of alleles (across all loci) was a key determinant of the accuracy of GSI. Therefore, we fixed K_{tot}, the total number of informative alleles in the simulation, at 256, and then varied L, the number of loci, in powers of two from 1 to 256. We assumed two populations with identical, uniform, allele frequencies. Thus, for a simulation with L loci, each locus had $k = 1 + K_{\text{tot}}/L$ alleles, each at a frequency of $1/k$. Five-hundred different baselines were simulated by sampling 144 individuals for each population from these uniform allele frequencies. For each baseline, a single mixture of $N = 400$ fish, all of them from population 1, was simulated and analyzed using the CV-ML, CV-SL, CV-GC, PB-R, and PB-NR methods. For each mixture, we recorded the average value, over individuals in the mixtures, of $\hat{T}_{j,1}$. We present the results in terms of the average value of $\hat{T}_{j,1}$ over the 500 replicate baselines. Because the two populations have identical allele frequencies, the expected value of $\hat{T}_{j,1}$ is 0.5, and any systematic departure from that is a sign of bias.

Analysis of empirical data

We use the coastwide Chinook microsatellite baseline developed by the PSC-funded GAPS consortium (version 2.1 of the baseline, unpublished data, obtained in 2006 from M. Banks, Coastal Oregon Marine Experiment Station, Hatfield Marine Science Center, Department of Fisheries and Wildlife, Oregon State University, Newport, OR 97365) as the

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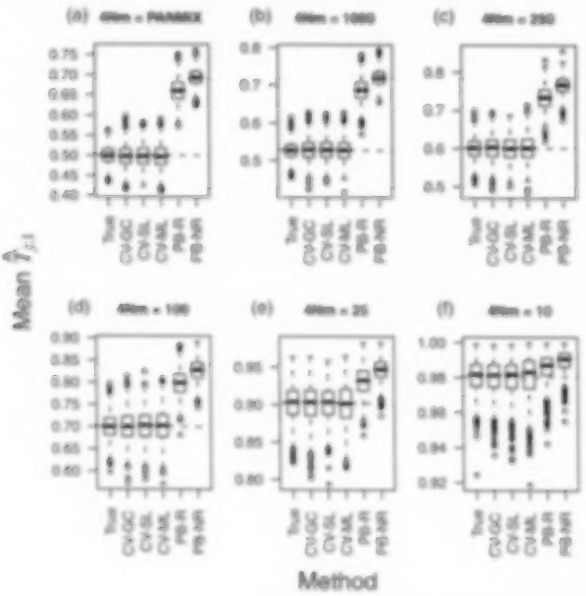

Fig. 2. Box plots of $\bar{T}_j$ simulated in five different ways. Bold lines are the medians, boxes span the interquartile range, whiskers extend to 1.5× the interquartile range beyond each box edge, and open circles denote data points falling beyond the whiskers. For each replicate, 400 fish from population 1 were simulated and the mean value of $\hat{T}_{j,1}$ over those 400 fish was computed. Each box plot summarizes the distribution of the mean of the $\hat{T}_{j,1}$ values over 1000 replicates. (*a*–*f*) Different migration rates, as shown in the title of each panel. The method producing each box plot appears below the *x* axis. In the "true" method, each mixture sample of 400 simulated fish is obtained by simulating additional leaves on the coalescent tree used to simulate the baseline samples. In addition to the bold line in the box plot, an extended broken line appears at the average $\hat{T}_{j,1}$ value obtained with the true method. Departures by the other methods from this line represent bias. In the other methods, each mixture sample of 400 fish is simulated by resampling from the baselines via either a parametric bootstrap method (PB-R, parametric bootstrap with baseline resampling; PB-NR, PB without baseline resampling) or a cross-validation method (CV-GC, cross-validation over gene copies; CV-SL, CV over single locus genotypes; CV-ML, CV over multilocus genotypes).

basis for two analyses to demonstrate practical consequences of using the conventional, biased method for predicting GSI accuracy. This baseline includes 22 231 fish from 166 Chinook salmon populations ranging from central California to Alaska (the Quinault Hatchery population was removed because of its small sample size). The baseline contains at least 144 fish from most of the 166 populations.

In the first analysis, we performed "100% mixture simulations" for each population in the baseline. In such a simulation, multiple mixture samples composed entirely of fish from a single population, say population *i*, are simulated and analyzed. The accuracy of GSI is assessed on the basis of how close the average value of $\bar{\pi}_i$ is to 100%. Such 100% mixture simulations may not be a particularly realistic way of assessing the utility of a set of markers for GSI, but they have been used numerous times in the literature (for example, see Seeb and Crane (1999), Smith et al. (2005*b*), Habicht et al. (2007), etc.). We simulated one-hundred 100% mixtures of size $N = 200$ for each of the 166 populations in the baseline. These mixtures were simulated using both the CV-GC and the PB-R methods. For each method, we recorded the mean over the 100 samples of $\hat{\pi}_i$. A separate set of simulations was performed with identical conditions except that each mixture was composed entirely of individuals from populations within a single one of 44 reporting groups that partition the 166 populations. Populations within each reporting group were assumed to contribute equally to the mixture. For the reporting group simulations, we recorded the mean over 100 replicates of the sum over populations *i* in the reporting group of $\hat{\pi}_i$.

In the second analysis, we simulated 2500 mixed-fishery samples of size 400 fish using both the CV-GC and the PB-R methods. The value of $\boldsymbol{\pi}$ used to simulate the mixture samples was set close to the mixing proportions estimated using GSI from 735 Chinook salmon sampled from the Monterey Bay recreational fishery in the spring of 2006 (Table 1). Each sample was analyzed using the entire coastwide Chinook baseline. We recorded the mean and mean squared error (MSE) over all 2500 samples of $\hat{\pi}_i$ for each population *i* represented in the mixture. Additionally, we assigned each fish in every simulated mixture to the population having highest posterior probability, approximated using the MLE, $\hat{\boldsymbol{\pi}}$. In other words, fish *j* was assigned to the population *i* that had the maximum value of

$$P_j = \frac{\hat{\pi}_i T_{j,i}}{\sum_k \hat{\pi}_k T_{j,k}}$$

We recorded the fraction of fish from each population assigned in this manner to the correct population.

Results

Simulation experiments

The results of our first simulation experiment demonstrate a profound bias in $\bar{\boldsymbol{T}}_j$ when using the PB-NR and PB-R methods and show that the cross-validation methods provide essentially unbiased values of $\bar{\boldsymbol{T}}_j$ (Fig. 2). Because this vector can be shown to be the sufficient statistic for estimating $\boldsymbol{\pi}$ using the conditional MLE method, bias in $\bar{\boldsymbol{T}}_j$ indicates a serious problem for any simulation procedure. The bias is most pronounced when the two simulated populations are panmictic. In that case, the expected value of $\bar{T}_{j,1}$ is, by a simple symmetry argument, 0.5, which is also the mean value obtained using the CV-ML, CV-SL, and CV-GC methods. In contrast, as expected for PB-NR, the distribution of $\bar{T}_{j,1}$ is extremely biased, having a mean close to 0.7. Interestingly, using resampled baselines does little to correct the bias: the PB-R method shows an average value of $\bar{T}_{j,1}$ of 0.66, far closer to the PB-NR value 0.7 than to the correct value of 0.5. As the migration rate decreases, the relative magnitude of the bias decreases, but the PB-NR and PB-R methods continue to deliver clearly biased values of $\bar{T}_{j,1}$ all the way up to $4N_em = 10$ (expected $F_{ST} \approx 0.09$). All the cross-validation methods appear to remain unbiased at all migration rates.

The impact of this bias on the estimation of $\boldsymbol{\pi}$ is pronounced (Fig. 3). Even when a mixture is composed entirely

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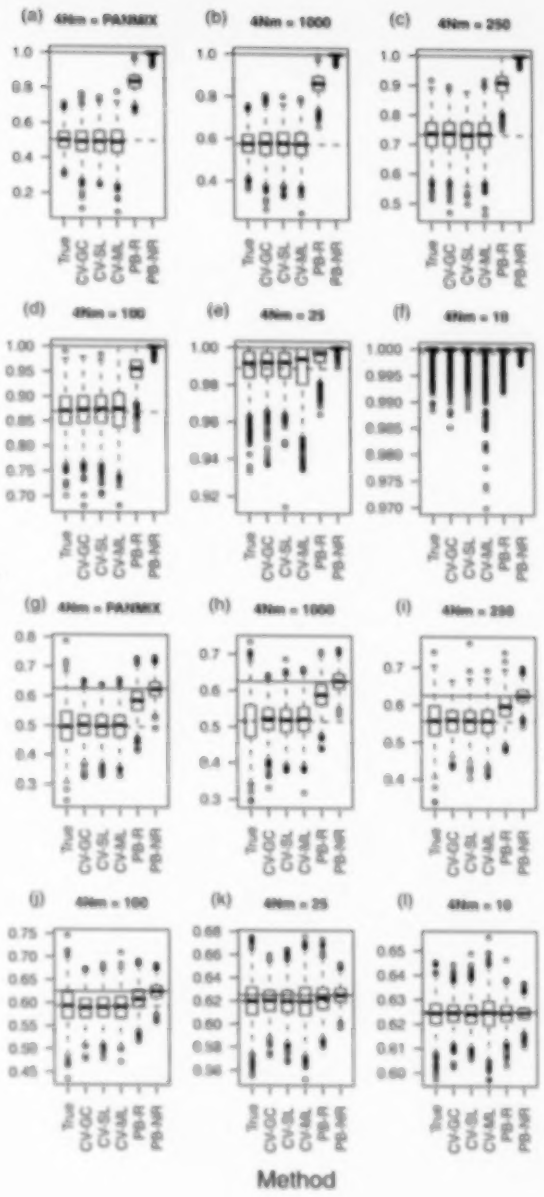

Fig. 3. Box plots of $\hat{\pi}_1$ in a two-population mixture model. Each box plot summarizes 1000 replicate simulations with $n_1 = n_2 = 144$ and $N = 400$. The actual proportion of fish from population 1 in each simulated mixture sample is represented by the horizontal, solid line (1.0 in panels *a–f* and 0.625 in panels *g–l*). The "true" box plot shows the true distribution of $\hat{\pi}_1$ obtained when the mixture sample is from the same population as the baseline sample but is taken separately from the baseline. The mean of this distribution is also denoted with a horizontal, broken line. The other box plots reflect mixtures obtained by resampling from the baselines. The distribution of $\hat{\pi}_1$ is notably biased for the PB-NR and PB-R methods. Such methods overestimate the accuracy of genetic stock identification (GSI). PB-R, parametric bootstrap with baseline resampling; PB-NR, PB without baseline resampling; CV-GC, cross-validation method over gene copies; CV-SL, CV over single locus genotypes; CV-ML, CV over multilocus genotypes.

of fish from population 1, if populations 1 and 2 are panmictic, then the true, expected value of $\hat{\pi}_1$ is 0.5. The average value of $\hat{\pi}_1$ obtained with the cross-validation methods is very close to 0.5. For the PB-R method, however, the average is 0.82. In this case, the bias inherent in $\hat{\boldsymbol{T}}_j$ using the PB-R method is magnified, creating an even more apparent bias in the estimates of $\hat{\pi}_1$. All the results in Fig. 3 show that the PB-R method provides biased estimates of π_1 at various migration rates and for two different true values of π_1, demonstrating that the conventional method overestimates, sometimes greatly so, the accuracy that can be expected from GSI given a particular baseline.

Though the mean values of $\bar{T}_{j,1}$ and $\hat{\pi}_1$ obtained using the cross-validation methods are very close to those obtained with the "true" method, it can be seen from the box plots that there are differences between the full distributions of $\bar{T}_{j,1}$ and $\hat{\pi}_1$ obtained by the different approaches. This is likely the case because $\hat{\boldsymbol{T}}_j$s simulated by the cross-validation methods are not conditionally independent given the baseline, as they are under the true model. Despite this non-independence, however, it appears that the cross-validation methods provide an adequate approximation to the true distribution.

The five-population simulations deliver results (Fig. 4) similar to the two-population simulations. Under panmixia, the true expected value of $\hat{\pi}_i$ is 0.2 (one divided by the number of populations), and in fact, the observed mean $\hat{\pi}_i$ of the "true" simulations (circles in Fig. 4) do fall on 0.2 for the panmictic simulations. The mean $\hat{\pi}_i$ from the CV-GC method is also close to 0.2; the slight discrepancy, where it appears, is due, most likely, to the means being based on only 25 replicates. The PB-R method, however, tends to produce estimates of $\hat{\pi}_i$ that are much closer to the value of π_i used to simulate the mixtures. As before, when the divergence between populations increases, the bias becomes less apparent: with $4Nm = 25$, the average $\hat{\pi}_i$ is close to the true value of π_i for all methods. This suggests that the bias of the PB-R method should not be extremely problematic if all populations are well diverged genetically. These simulations, like the previous ones, confirm that the PB-R method yields consistently upward-biased estimates of GSI accuracy.

In our third simulation experiment, we found that the total number of alleles exerts a strong influence on the bias of the PB-R method (Fig. 5). The distribution of those alleles into a large or small number of loci had a secondary influence on the bias. For example, the degree of bias, measured in terms of mean $\bar{T}_{j,1}$, was nearly equal whether using 256 diallelic loci or 8 loci with 33 alleles each. (Because the frequency of one allele at each locus is completely determined by the remaining alleles, each diallelic locus has one independent allele and each locus with 33 alleles has 32 alleles, which, though not technically independent, are often referred to in the literature as "independent" alleles. Thus, 256 diallelic loci and 8 loci with 33 alleles have the same number of "independent" alleles.) Only when the number of alleles per locus approaches 64 does there seem to be a noticeable increase in bias resulting from the very low allele frequen-

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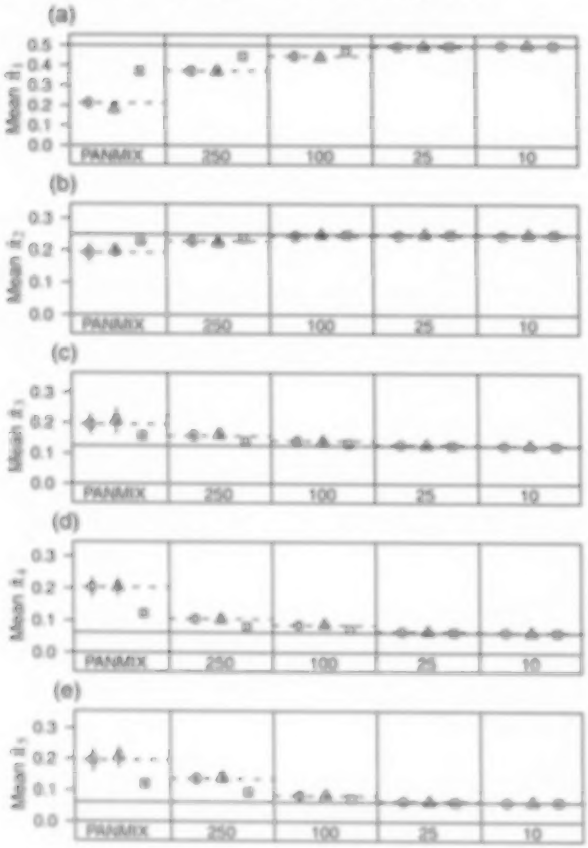
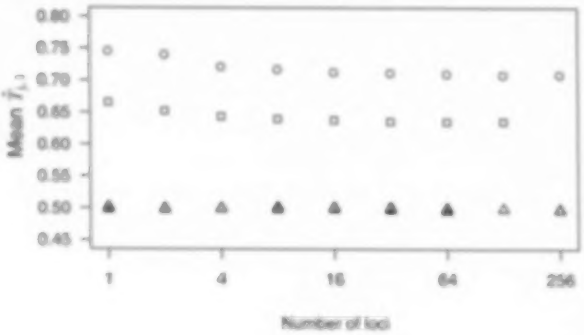
Fig. 4. Mean $\hat{\pi}$ in a simulation of five populations (*a*–*e*, populations 1–5, respectively). The true proportion that each population contributes to the mixture is shown by a horizontal, solid line. Results from simulations using different migration rates are designated by the migration rates listed at the bottom of each panel. Circles represent the mean of the average $\hat{\pi}$ expected if mixture samples are separate from baseline samples. These reflect the true accuracy that can be expected of genetic stock identification (GSI) given a baseline. Additionally, a horizontal, broken line is drawn at the level of this true expected $\hat{\pi}$. Triangles indicate the results for the CV-GC (cross-validation over gene copies) method. The open squares indicate the results for the PB-R (parametric bootstrap with baseline resampling) method. Vertical bars denote the standard deviation of the means. The CV-GC values correspond closely to the true, expected accuracy of GSI. The PB-R values are biased toward the true value of π, suggesting greater accuracy than is truly available from a given baseline. The differences between the methods are minimal at low migration rates (high genetic divergence) but are remarkable at high migration rates (low divergence).



cies, and even this increase is minor. The results also show that with more data (i.e., more alleles in total), the bias increases. In other words, as more and more genetic markers become available and are applied to resolve mixtures of closely related populations, it becomes increasingly vital that an unbiased method of GSI accuracy prediction be used. Regardless of the number of alleles or loci in this simulation, all the cross-validation methods yielded unbiased

Fig. 5. Effect of the number of alleles per locus on the magnitude of the bias. Total number of alleles is constant, but number of loci changes. Cross-validation methods (CV-GC, CV over gene copies; CV-SL, CV over single locus genotypes; CV-ML, CV over multilocus genotypes) with 128 and 256 alleles are all denoted by open triangles. Parametric bootstrap method with baseline resampling (PB-R) with 128 and 256 alleles are denoted by open squares and open circles, respectively. In a two-population scenario, the amount of bias in the PB-R method is shown here to be roughly constant, whether alleles are apportioned into just a few loci, each with many alleles, or into many loci, each with only two alleles.



values of $\hat{T}_{1,1}$. (Recall that, by symmetry, the true expectation of $\hat{T}_{1,1}$ is 0.5.)

Analysis of empirical data

The simulations described above established that the cross-validation methods provide largely unbiased estimates of GSI accuracy. Now, the results obtained using the CV-GC and PB-R methods applied to real data give us a sometimes alarming picture of the practical implications of the PB-R method's bias. This is plainly apparent in the 100% simulated mixtures of populations in the GAPS baseline (Fig. 6*a*). Using the PB-R method, only 17 of 166 populations had a mean $\hat{\pi}_i < 0.8$. By contrast, the CV-GC method reveals that fully 78 of 166 populations have mean $\hat{\pi}_i$ values less than 0.8. Many populations having mean $\hat{\pi}_i \approx 0.85$ under the PB-R method have mean $\hat{\pi}_i$ close to 0.6 using the CV-GC method. For all those populations, the predicted accuracy using PB-R is biased upward by some 40% from its unbiased value obtained using CV-GC. When focusing on reporting units, the bias appears to be not as great because, overall, accuracy is higher for estimating contributions of reporting units than for estimating contributions of their constituent populations. Nonetheless, there is some bias apparent (Fig. 6*b*). When using PB-R, none of the 44 reporting units (except for one, composed of a single population) has mean π values < 0.9, whereas when using the CV-GC method, 4 of them do.

The fact that the bias of the PB-R method is of less consequence when genetic differentiation is substantial between populations was confirmed in our simulations mimicking the Monterey Bay recreational fishery (Table 1). There were no clear, systematic differences in the mean estimates of π between the CV-GC and the PB-R methods relative to the value of π used to drive the simulation. In this case, the lack of apparent bias in PB-R is not unexpected: even when

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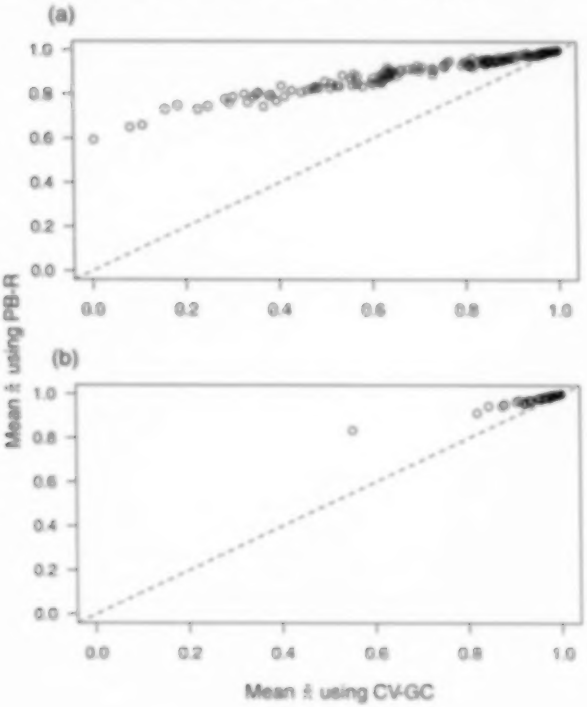
Fig. 6. One-hundred percent mixture results. (*a*) Each circle represents a single one of 166 populations in the coastwide Chinook baseline. (*b*) Each circle represents one of 44 regions (or reporting units) into which the 166 populations fall. The position along the *x* axis indicates the mean over 100 simulations of the proportion of that population (or region) estimated from a mixture sample composed exclusively of members from that single population (or region) simulated using the CV-GC method (cross validation over gene copies). The *y* axis indicates the results using the parametric bootstrap method with baseline resampling (PB-R). The broken line falls along $y = x$. The PB-R method is biased upward relative to the CV-GC method, overestimating genetic stock identification (GSI) accuracy (greatly so in the case of some individual populations).



using the CV-GC method, the mean $\hat{\pi}_i$ is close to the true value of π_i for all the populations. If you are getting an excellent estimate from an unbiased method already, then the bias of the PB-R method cannot contribute much spurious, additional accuracy. Additionally, the MSE of the estimates were not substantially different between the two methods. This, also, is to be expected as most of the MSE in this case results from the multinomial variance associated with the number of fish from each population appearing in each simulated mixture sample, and not from the inaccuracy of genetic discrimination between the populations.

Though there was no discernible difference between using CV-GC and PB-R in the estimation of $\hat{\pi}$ in the Monterey Bay Fishery simulations, there was a marked difference in the observed rate of correct assignment of individual fish to their population of origin (see Individual assignment columns in Table 1). The fraction of correctly assigned fish using the PB-R method was always biased high relative to the fraction using the CV-GC method. For some populations, this bias was very pronounced. For example, the estimated rate of correct allocation of salmon from Stanislaus River and Butte Creek Fall, using PB-R, is over three times too high. For applications requiring a careful assessment of the accuracy of assignment of individual fish from mixed fisheries, it is critically important to use an unbiased method like one of the cross-validation methods in preference to the PB-R method.

Discussion

In this paper, we have demonstrated that the conventional simulation method, which we call the PB-R method, invariably provides a biased, overly optimistic prediction of the accuracy achievable with genetic stock identification. The PB-R simulation method involves resampling from the observed baseline to produce a simulated mixture and a simulated baseline that is used to analyze the simulated mixture. It has been widely appreciated that the PB-NR method, analyzing the simulated mixture with the observed baseline, might yield biased results, and we have shown this to be the case. However, for over 20 years, it seems to have been commonly believed that resampling the baseline as in the PB-R method will minimize or eliminate that bias; it is clear from our computer simulations that it does not.

We have also described and implemented a method that provides essentially unbiased esimates of GSI accuracy. The three versions of this method (CV-ML, CV-SL, and CV-GC) are all based on the leave-one-out, cross-validation procedure. Using simulated data, we first confirmed that our cross-validation methods give essentially unbiased results. Then, by applying the PB-R and CV-GC methods to empirical data sets, we were able to assess the practical consequences of the PB-R method's bias.

We wish to stress that our method yields essentially unbiased estimates of GSI accuracy only within the confines of the asssumptions of the model, and particularly the assumption that all populations in the mixture are accurately represented in the baseline. Clearly, if individuals will appear in the mixture from populations that do not occur in the baseline, or if there is temporal or spatial structure in the baseline populations that is not well represented by the baseline sample, then not even the CV-GC, CV-SL, or CV-ML methods will reflect the accuracy that you can expect in performing GSI. It would seem that there is no way to systematically and correctly account for the possibility of individuals from unsampled populations in the mixture.

There is little apparent difference in results for the three cross-validation methods that we tried, but these differences might be larger under certain conditions. For example, CV-ML has the advantage that if some loci (or pairs of loci) are out of Hardy–Weinberg (or linkage) equilibrium, that will be reflected in the simulated genotypes; in addition, this option might best reflect the distribution of missing data. On the other hand, CV-SL might be preferable if individual loci show Hardy–Weinberg departures but pairs of loci are in linkage equilibrium. Finally, CV-GC is perhaps the most flexible option, as it can be used when only allele frequencies (and not genotypes) are available, and the space of possible simulated genotypes is largest with that option.

To facilitate analysis of the large number of simulated

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data sets, we only evaluated results for the conditional GSI method. Although the unconditional GSI and Bayesian methods have some potential advantages for genetic mixture analysis, as currently implemented, they are not designed to deal with the problem of inflated F'_{ST} in simulated mixtures due to baseline sampling error discussed in this paper. Therefore, we would expect the same general patterns of bias from these methods as we found for conditional GSI.

The impact of the bias is greatest in situations involving closely related populations, and the extent of bias increases as more loci and alleles are added to the data set. Both of these observations make sense intuitively based on the concepts of signal-to-noise ratio and statistical power. In closely related populations, the noise (spurious divergence due to sampling error) is large relative to the true population divergence (signal), and this exacerbates the bias. For any given inflated value of F'_{ST}, the apparent (but inflated) power to resolve the mixture increases as more loci and more alleles are used. Further simulations (not shown) show that this latter effect occurs whether the genetic markers in question are microsatellites or SNPs.

In the fisheries literature, populations yielding an average estimated mixture proportion of 90% from 100% mixture simulations are often considered highly identifiable in mixed fisheries (Smith et al. 2005*b*). Using the essentially unbiased CV-GC method, only 54 of the 166 populations in the GAPS data set would be considered highly identifiable. Using the traditional PB-R simulations, however, 110 populations in the GAPS data set exceeded this highly identifiable threshold. Thus, in the case of the GAPS data, the PB-R method has misclassified more than half of the 110 populations placed in the highly identifiable category. We expect that most fisheries managers will find this degree of bias in the PB-R method unacceptable. One-hundred percent mixture simulations, using the PB-R method and typically implemented in SPAM (Alaska Department of Fish and Game 2000) or GMA (Kalinowski 2003), have been employed in a great number of studies. Unfortunately, our work indicates that re-evaluation of those past results may be warranted.

When inference for individual populations is not critical and similar populations can be aggregated into larger reporting units (collections of multiple populations), the consequences of the PB-R method's bias are not so dramatic. This occurs because the genetic divergence between members of different reporting units often is large enough that the additional bias from using the PB-R method is relatively less noticeable. Our results show that although there is still a consistent bias in the 100% mixture results for 44 reporting units, the relative magnitude of the bias is not nearly so great as with individual populations.

When multiple populations contribute to a mixture, the effects of the PB-R method's bias can be more difficult to predict. This is apparent from our simulated Monterey Bay Recreational Fishery samples. In this case, the PB-R and the CV-GC methods delivered similar estimates of the accuracy with which mixing proportions of individual populations can be estimated (i.e., the means and the mean-squared errors from both methods were roughly comparable). This is likely because the actual numbers of fish from each population in these simulated mixture samples were themselves random variables (with means equal to N times the true π), and the variance associated with each one resulting from this random variation may have been large enough to obscure the differences between the PB-R and the CV-GC methods. On the other hand, if one intends to use genetic data not only for estimating π, but also for allocating individual fish to populations, then the PB-R method still provides strongly biased results. In the Monterey Bay example, when individuals were assigned to the population having highest posterior probability, the rate of correct assignment predicted by the PB-R method was, for some populations, over three times greater than the unbiased prediction from the CV-GC method.

Our results apply to simulations in which hypothetical mixture samples are simulated using the baseline as input. When in possession of both a baseline sample and a separate sample from a mixed fishery, the sampling distribution of $\hat{\pi}$ is sometimes estimated by repeatedly bootstrap resampling a new mixture sample from the real mixture sample and a new baseline sample from the observed baseline sample and then estimating π from the bootstrapped samples (Fournier et al. 1984). Because this procedure does not induce a positive correlation (with respect to the true population allele frequencies) between the bootstrapped mixtures and the baselines, it should not suffer from the same degree of bias as the PB-R method. In fact, this is apparent by comparing the OBSERVED, SIMULATE, and BOOTSTRAP columns of tables 10.2 and 10.3 in Pella and Milner (1987, p. 254). It should be kept in mind, however, that such a bootstrapping scheme yields only the sampling distribution of $\hat{\pi}$, which may not reflect the range of possible true values of π that could yield such estimates, especially when closely related populations are present in the baseline.

We introduced this problem in terms of the biased value of F_{ST} between populations obtained when the effect of sampling is not taken into account. If one desires merely an unbiased estimator of F_{ST}, then such an estimator is available using an analysis of variance approach (Weir and Cockerham 1984). On the basis of such an unbiased estimate of F_{ST}, it would be possible to simulate genes from two populations, adjusting the results so as to achieve samples having properties that would be expected of two populations diverged by an amount F_{ST} rather than by F'_{ST} (see Introduction). Such maneuvers might allow for simulations of mixtures and baselines that would provide a less biased (than PB-R) prediction of GSI accuracy, using an approach that is of an entirely different character than ours. In fact, such a method was used to parametrize simulations of spring- and fall-run Chinook salmon in the Trinity River (Kinziger et al. 2008) designed to help interpret the results of the program *structure* (Pritchard et al. 2000). Though such an approach is useful when only two populations are involved, its implementation becomes more complicated when the baseline includes a large number of populations at widely varying degrees of divergence. A related approach would involve using estimates of baseline frequencies that were adjusted by some shrinkage toward a grand mean allele frequency. For predicting GSI accuracy, it seems unlikely that such shrinkage methods would be as elegant and simple to implement as the cross-validation method; however, it could prove very useful in some contexts.

The bias effect we have described might not be limited

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merely to genetic stock identification in fisheries; it could also confound other accuracy estimation problems in molecular ecology. One example occurs with Bayesian, model-based clustering approaches like those implemented in *structure* (Pritchard et al. 2000), *NewHybrids* (Anderson and Thompson 2002), and *BayesAss+* (Wilson and Rannala 2003). With closely related populations, it may be difficult to interpret the output from these programs, and it is thus becoming increasingly common to run the programs multiple times on data simulated to look like the original data set and, from the ensemble of outputs, arrive at an interpretation of the original results. For example, Nielsen et al. (2003) take this approach using the program *structure* to argue that there is an Atlantic cod hybrid zone. We believe that doing this type of simulation is an important step in applying such Bayesian clustering methods; however, our current work demonstrates that particular care must be taken in choosing how to perform the simulations. For example, naively simulating new data sets from the posterior mean allele frequencies in the clusters suffers from the same problems as the PB-R method and could lead one to conclude that more power is available for resolving clusters than really exists. It might be possible, however, to design such simulations using the same principles of leave-one-out cross validation as applied here. We are currently investigating the potential for this in the context of Bayesian model checking and sensitivity analysis executed during the run time of the Markov chain Monte Carlo algorithm of each program.

We foresee that more and more genetic markers will become available, and the demands placed on them for fisheries management will only increase. Managers will likely seek resolution between increasingly closely related populations, and applications for allocation of individual fish will continue to develop in fisheries. In such a climate, it is important to have accurate methods for assessing statistical power for GSI. We suggest that the software packages currently in use be updated to include, as the default option, at least one of the cross-validation methods described here. ONCOR, a Windows-based program, for implementing the simulations described here is available at http://www.montana.edu/kalinowski. Another program, *gsi_sim*, with a command line interface suitable for Unix-like operating systems is available at http://swfsc.noaa.gov/staff.aspx?id=740.

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Evaluation of the human health threat associated with the hepatotoxin microcystin in the muscle and liver tissues of yellow perch (*Perca flavescens*)

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Abstract: During the summer of 2006, the western basin of Lake Erie experienced a bloom of the toxigenic cyanobacterium *Microcystis aeruginosa*. Across 11 sites, intracellular, particulate-bound microcystin levels in the seston increased to levels that exceeded World Health Organization guidelines for drinking water exposure (1 μg toxin·L^{-1}). In contrast, toxin concentrations in yellow perch (*Perca flavescens*) muscle tissue (n = 68) declined from June to August, were negatively related to algal toxin levels, and never exceeded a conservative chronic exposure concentration estimated using proposed United States Environmental Protection Agency (US EPA) guidelines. Microcystin concentrations in yellow perch liver exceeded US EPA chronic exposure guidelines, were on average 125 times higher than muscle toxin concentrations per unit dry weight, and varied little throughout the summer. With current guidelines, humans do not appear to be at risk when consuming the muscle tissue of Lake Erie yellow perch collected during large-scale cyanobacterial blooms. However, this study highlights the need for a better understanding of the trophic transfer of cyanobacterial toxins through aquatic food webs in diverse ecosystems with an emphasis on understanding if these compounds could accumulate sufficiently to affect human health.

Résumé : Durant l'été 2006, le bassin occidental du lac Érié a connu une floraison de la cyanobactérie toxinogène, *Microcystis aeruginosa*. Dans 11 sites, les concentrations de microcystine intracellulaire liée aux particules dans le seston ont atteint des niveaux supérieurs aux normes de l'Organisation mondiale de la santé pour l'eau potable (1 μg toxine·L^{-1}). En revanche, les concentrations de toxines dans le tissu musculaire de la perchaude (*Perca flavescens*) (n = 68) ont diminué de juin à août et étaient en corrélation négative avec les concentrations de toxines dans les algues; elles n'ont jamais dépassé une concentration conservatrice d'exposition chronique estimée à partir des recommandations de l'Agence de protection de l'environnement des États-Unis (US EPA). Les concentrations de microcystine dans le foie des perchaudes dépassaient les concentrations mentionnées dans les recommandations sur l'exposition chronique de US EPA; elles étaient en moyenne 125 fois plus élevées que les concentrations de toxines dans le tissu musculaire par unité de masse sèche et elles ont peu varié au cours de l'été. D'après les recommandations actuelles, les humains ne semblent pas courir de risque en consommant du tissu musculaire de perchaudes du lac Érié capturées durant les floraisons à grande échelle de cyanobactéries. Cependant, notre étude met en évidence la nécessité de mieux comprendre le transfert trophique des toxines des cyanobactéries dans les réseaux alimentaires aquatiques dans les divers écosystèmes et, particulièrement, de savoir si ces composés peuvent s'accumuler suffisamment pour affecter la santé humaine.

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Introduction

Noxious cyanobacterial blooms are global phenomena that occur in freshwater and estuarine habitats in response to increased nutrient loading, alterations in nutrient stoichiometry, food-web manipulations, and biotic invasions (Schindler 1974; Paerl 1997; Sarnelle et al. 2005). Common cyanobacterial genera associated with harmful freshwater algal blooms include *Anabaena*, *Aphanizomenon*, *Cylindrospermopsis*, *Microcystis*, and *Oscillatoria* (Carmichael 1992; Chorus and Bartram 1999; Zurawell et al. 2005). A significant consequence of cyanobacterial blooms is that intracellular toxins, such as the hepatotoxin microcystin or the neurotoxin anatoxin-*a*, released from these events can adversely impact aquatic systems used for recreation and drinking water (Francis 1878; Carmichael et al. 2001; Wiegand and Pflugmacher 2005). Although the ecological function of cyanotoxins is unresolved (Rantala et al. 2004; Wilson et al. 2006*a*; Schatz et al. 2007), it is clear that microcystins, a class of cyclic heptapeptides that are produced by several bloom-forming, cyanobacterial genera (e.g., *Microcystis*), reduce population growth in some zooplankton when consumed (Wilson and Hay 2007), inhibit protein phosphatases (An and Carmichael 1994), and promote liver tumors in mammals (Carmichael 1994; Watanabe et al. 1996; Codd et al. 2005). Consequently, to protect human health, the World Health Organization (WHO) has recommended that the maximum threshold of total dissolved and particulate microcystins in drinking water be set at 1 μg toxin-L^{-1} (Chorus and Bartram 1999). Microcystin concentrations in drinking water supplies that exceed this level may negatively impact human health and should concern water quality managers.

Cyanotoxin exposure for humans includes ingestion or inhalation of water containing toxin-producing cyanobacteria or dissolved compounds recently released by senescent cells (Christoffersen 1996; Sivonen 1996; Chorus and Bartram 1999). Consumption of fish containing cyanobacterial toxins represents a poorly studied, but potentially important, mechanism for the ingestion of harmful cyanotoxins by humans. Fresh- and brackish-water fishes are known to accumulate cyanotoxins in tissues, including muscle, viscera, and liver (Kotak et al. 1996; Ibelings et al. 2005; Gkelis et al. 2006). A recent study showed that microcystin accumulation rates in muscle and liver tissues are directly proportional to ingestion rates for at least one species of fish (i.e., Nile tilapia (*Oreochromis niloticus*); Zhao et al. 2006). Concentrations of microcystin are routinely shown to be much higher in fish liver than in other tissues (Magalhães et al. 2001; Xie et al. 2005; Chen et al. 2007), which is not surprising given the hepatotoxic properties of microcystins. In addition, although rarely reported in equivalent units, microcystin concentrations in fish liver tend to be orders of magnitude lower than concentrations in seston when collected from the same habitat (Ibelings et al. 2005). Possible explanations for this difference in tissue toxin concentrations include detoxification by higher organisms, microbial degradation, low biomagnifications, complex food-web transfer dynamics, and incomplete extraction procedures of cyanobacterial toxins (Christoffersen 1996; Sivonen 1996; Ibelings et al. 2005). Fewer studies have quantified microcystin levels in fish muscle, i.e., the tissue more generally consumed by humans (but see Sipiä et al. 2001; Magalhães et al. 2003; Xie et al. 2005). For the Great Lakes, we are unaware of any published study reporting microcystin levels in the muscle tissue of a sportfish, although toxin data for muscle tissues do exist for round goby (*Neogobius melanostomus*) collected in Hamilton Harbor, Lake Ontario (Murphy et al. 2003). Further, Babcock-Jackson (2000) reported mean gut (46 ng toxin-(g wet weight)$^{-1}$) and liver (127 ng toxin-(g wet weight)$^{-1}$) microcystin concentrations for fishes collected from western Lake Erie in August 1998.

The study presented herein focuses on microcystin accumulation in muscle and liver tissues of commercially and recreationally important yellow perch (*Perca flavescens*) from western Lake Erie. In Lake Erie, yellow perch are omnivorous, with diet patterns varying seasonally and ontogenetically (Tyson and Knight 2001). Similar to most fish species, as yellow perch grow in size, they shift from consuming mostly small-bodied prey (e.g., small zooplankton) to consuming larger prey (e.g., large zooplankton, benthic invertebrates, and fish; Hayes and Taylor 1990; Wu and Culver 1992). In western Lake Erie, benthic invertebrates consistently constitute the primary diet category (by weight) for age-1 and older yellow perch, whereas zooplankton (June–July) and fish (August–September) appear to be seasonally important (Tyson and Knight 2001). Thus it is feasible that seasonal microcystin accumulation by yellow perch will mirror such seasonal shifts in diet.

Our study represents the first, large-scale effort to document concentrations of microcystin in a Great Lakes fish species widely consumed by humans. Specifically, we aimed to elucidate potential health risks for humans who consume yellow perch containing this toxic intracellular compound. To this end, microcystin concentrations were determined for lake seston and the liver and muscle tissues of yellow perch, collected monthly (June to August 2006) across 11 sites throughout the western basin of Lake Erie. This sampling period coincided with the development of a massive cyanobacterial bloom (chlorophyll *a* range = 1–47 μg chlorophyll-L^{-1}) that was dominated by the toxigenic cyanobacterium *Microcystis aeruginosa*. We related yellow perch tissue microcystin concentrations to toxin levels in their environment to identify possible mechanisms of microcystin uptake (i.e., through food-web interactions) with an ultimate goal of understanding the threat that these fish pose to human health.

Materials and methods

Seston and yellow perch were collected from 11 sites (maximum depth range = 5.5–10.4 m; Table 1) throughout the western basin of Lake Erie (Fig. 1) on 22 June 2006, 18 July 2006, and 22 August 2006. Coincident with these collections, temperature, dissolved oxygen concentration (DO), conductivity, and pH were measured throughout the water column with an YSI sonde (model 6600EDS-M, YSI Incorporated, Yellow Springs, Ohio). Depth-integrated seston samples were collected with a tube sampler (3 cm diameter opening) from the surface to near the lake bottom and were then stored in a sealed, 10 L plastic cubitainer on ice. Yellow perch were collected with 10 min bottom trawls at each site and stored in plastic bags on ice until returning to the lab, where they were stored in a freezer (–20 °C).

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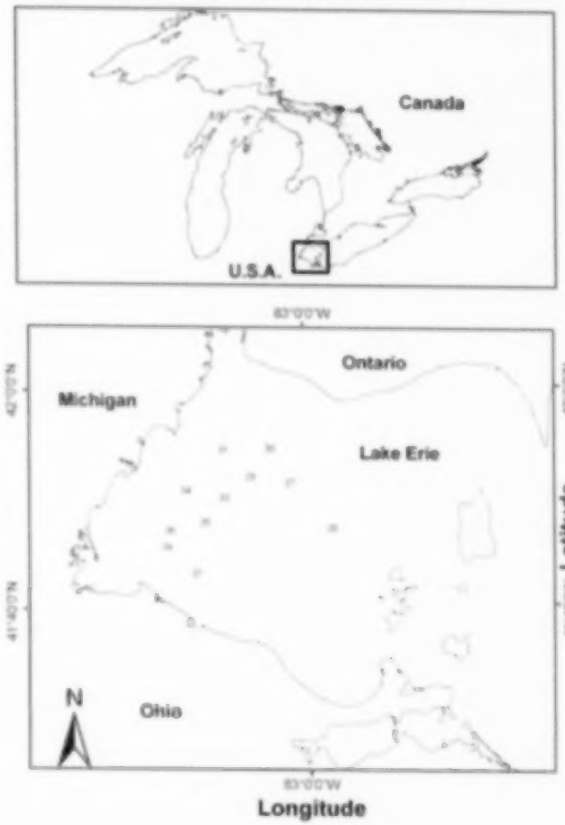
Table 1. Sample site locations and maximum depths (m) located in the western basin of Lake Erie.

Site	Latitude (N)	Longitude (W)	Depth (m)
26	41°46.1214′	82°58.2574′	10.4
27	41°50.2954′	83°03.3095′	10.1
29	41°50.8489′	83°08.1351′	9.8
30	41°53.4776′	83°05.7531′	9.5
31	41°53.4135′	83°11.3645′	8.8
33	41°48.9377′	83°11.3832′	8.2
34	41°49.6421′	83°16.0615′	7.9
35	41°46.7397′	83°13.7556′	8.2
36	41°46.0468′	83°18.0830′	6.7
37	41°42.0962′	83°14.9052′	7.3
39	41°44.5367′	83°18.4618′	5.5

In the laboratory, well-mixed 1000 mL seston samples were collected on filters (Whatman GF/F) for seston dry weight, chlorophyll, and microcystin content. Seston dry weight was determined after weighing tared and dried filters with collected seston (model AT250, Mettler-Toledo Inc., Columbus, Ohio). Chlorophyll was measured after a 24 h dark extraction at 4 °C in 95% ethanol via fluorometry (model 10AU, Turner Designs, Inc., Sunnyvale, Calif.). Intracellular microcystins in the seston were extracted twice (60 min·extraction^{-1}) from each seston sample using 15 mL 75% aqueous methanol. Concentrated acetic acid (60 µL) was added to the second extraction to lower solvent pH and to enhance extraction of microcystin. The extracts from each sample were collected via centrifugation, pooled, filtered through a glass-fiber prefilter (Nalgene, Nalge Nunc International Corp., Rochester, N.Y.), dried using vacuum evaporation, redissolved in water using sonication, and analyzed using enzyme-linked immunsorbant assay (ELISA; model EP-022, EnviroLogix Inc., Portland, Maine; An and Carmichael 1994). Using this protocol, seston microcystin concentrations can be quantified as microcystins per volume (ng toxin·L^{-1}), per dry weight (ng toxin·(g dry weight)$^{-1}$), and per unit chlorophyll (ng toxin·(µg chlorophyll)$^{-1}$). The volume metric provides a way to compare toxin abundances across water samples relative to the same volume of water filtered. The dry weight metric allows for cross-sample comparisons related to the amount of organic and inorganic material collected on the filter. The chlorophyll metric removes variance associated with inorganic, non-autotrophic material on filters and provides a technique to standardize toxin concentrations relative to the abundance of phytoplankton in the water sample. Because algal communities are comprised of mixtures of phytoplankton species that are able (i.e., cyanobacteria) or unable (e.g., green algae, diatoms, dinoflagellates) to produce microcystins, toxin concentrations relative to chlorophyll abundance are conservative and may be higher if only toxigenic cyanobacterial chlorophyll concentrations were known.

In the laboratory, whole fish were thawed and measured (total length and wet weight), and a sample of dorsal muscle tissue and the entire liver were collected, dried, and weighed. Microcystins were extracted twice from each tissue sample using 75% methanol. The first extraction (20 mL solvent) lasted for 2 h and included mechanical homogenization. The second extraction (30 mL solvent) included an addition of acetic acid (60 µL) and lasted for 24 h while the samples were stored at 4 °C in a dark environment. Extracts were collected via centrifugation, pooled, filtered through a glass-fiber prefilter (Nalge Nunc International Corp.) to remove particles, dried using vacuum evaporation, redissolved in water using sonication, and analyzed using ELISA.

Fig. 1. The Laurentian Great Lakes (upper map) and sampling sites in western basin Lake Erie (lower map). Sampling dates: 22 June 2006, 18 July 2006, and 22 August 2006. Latitude, longitude, and maximum depths for the sites can be found in Table 1.



The detection limit for microcystins present in extracts was 0.16 ng·mL^{-1}, and no microcystins were detected in filter and tissue blank samples. Microcystin recoveries of spiked samples were 92% for seston filter samples and 64% for liver and muscle tissue samples. Our recovery rates are consistent with recoveries from several past studies that extracted microcystins in lake water (91%–106% (Cong et al. 2006), 92%–111% (Wang et al. 2007)) and fish tissues (68% (Ernst et al. 2005), 68% (Ibelings et al. 2005), 63%–71% (Chen et al. 2007)). All reported toxin data, uncorrected for recovery rates, are for freely available toxins and do not include covalently bound microcystins. The concentrations are expressed as microcystin-LR equivalents based on the ELISA protocol (EnviroLogix Inc.); however, it is important to note that quantification is not consistent among microcystin variants. The ELISA kit used in this study (model EP-022, EnviroLogix Inc.) was developed to quan-

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Table 2. Mean chemical and physical characteristics of surface and bottom waters across 11 sites (see Fig. 1 and Table 1) in the western basin of Lake Erie sampled in the summer of 2006.

Date	Sample location	Statistic	Temperature (°C)	Conductivity ($\mu S \cdot cm^{-1}$)	DO ($mg \cdot L^{-1}$)	pH
22 June	Surface	Mean	22.45	257.09	8.49	7.99
		SE	0.19	7.44	0.03	0.02
		Minimum	21.35	219.00	8.32	7.89
		Maximum	23.66	301.00	8.69	8.12
22 June	Bottom	Mean	21.80	251.00	8.29	7.93
		SE	0.08	6.92	0.03	0.03
		Minimum	21.28	219.00	8.18	7.81
		Maximum	22.13	296.00	8.57	8.11
18 July	Surface	Mean	25.58	259.27	7.82	8.39
		SE	0.20	10.61	0.10	0.06
		Minimum	24.71	220.00	7.10	8.14
		Maximum	26.55	330.00	8.22	8.76
18 July	Bottom	Mean	25.34	276.27	8.76	8.26
		SE	0.11	17.15	0.17	0.05
		Minimum	24.84	224.00	8.31	7.80
		Maximum	25.85	360.00	9.93	8.43
22 August	Surface	Mean	25.34	236.00	8.65	8.46
		SE	0.48	25.60	0.04	0.06
		Minimum	23.63	3.00	8.33	8.32
		Maximum	27.95	330.00	8.86	9.03
22 August	Bottom	Mean	23.96	255.09	9.30	8.31
		SE	0.07	11.64	0.16	0.04
		Minimum	23.57	217.00	8.78	8.09
		Maximum	24.35	317.00	10.31	8.56

Note: Sample size for all measurements is 11. DO, dissolved oxygen; SE, standard error.

tify microcystin-LR and thus was most sensitive for this variant. Microcystin-LR is the most toxic variant (Carmichael 1992) and the most common form found in the western basin of Lake Erie (Dyble et al. 2008). Thus, although our measurments may be slightly conservative, our results should be indicative of the relative human health threat that microcystins pose to humans consuming yellow perch tissue. Regarding toxicity due to protein-phosphatase inhibition (the primary mode of toxicity for microcystins towards mammals; An and Carmichael 1994), ELISA does not measure phosphatase inhibition directly, but does provide correlative data associated with real cyanobacterial toxicity as toxin abundance and phosphatase inhibition are highly correlated (Fastner et al. 2002).

Gut contents were analyzed for a subset of the yellow perch collected in June 2006 (n = 17), July 2006 (n = 20), and August 2006 (n = 16) that was also analyzed for tissue microcystins. Stomachs were removed from thawed fish and preserved in 95% ethanol. Gut contents were determined via microscopy and grouped into the following categories: zooplankton, benthic invertebrates, fish, and unidentified. Gut samples were then dried at 70 °C and weighed to determine dry mass.

Differences in seston and tissue toxin levels were compared among sites and collection months via one-way analysis of variance (ANOVA). Unequal sample sizes precluded the use of two-way ANOVA for toxin abundance in yellow perch muscle and liver tissues across sites and time. Pearson's product moment correlations were used to evaluate associations among algal abundance, perch diet composition, and algal and tissue microcystin concentrations. Data were log- or arcsin-transformed as needed to conform to assumptions of parametric statistics. Nonparametric Kruskal–Wallis analyses were conducted when simple transformations failed to parameterize data. All analyses were performed with Systat 11 (Systat Software, Inc. 2004).

Results

Chemical and physical measures suggested that the western basin of Lake Erie was moderately well mixed from June to August 2006 (Table 2). Mean temperatures across all 11 sites ranged from 22.5 to 25.6 °C at the surface and from 21.8 to 25.3 °C at the lake bottom (Table 2). Conductivity and pH varied little over time (ranges 236–277 and 7.93–8.46 $\mu S \cdot cm^{-1}$, respectively), and the surface and bottom waters throughout the western basin of Lake Erie were well oxygenated (range 7.82–9.30 $mg \cdot L^{-1}$; Table 2).

Algal abundance (measured as chlorophyll a) ranged from 1 to 47 μg chlorophyll$\cdot L^{-1}$ among 11 sites throughout the western basin of Lake Erie during the summer of 2006. Chlorophyll concentration increased throughout the summer (June range 1.03–5.93 μg chlorophyll$\cdot L^{-1}$; July range 2.91–46.95 μg chlorophyll$\cdot L^{-1}$; August range 6.01–28.85 μg chlorophyll$\cdot L^{-1}$) and varied significantly over time (ANOVA, p = 0.016). Microscopic observations confirmed that the dominant phytoplankter of the summer algal community was *M. aeruginosa*. Seston microcystin concentra-

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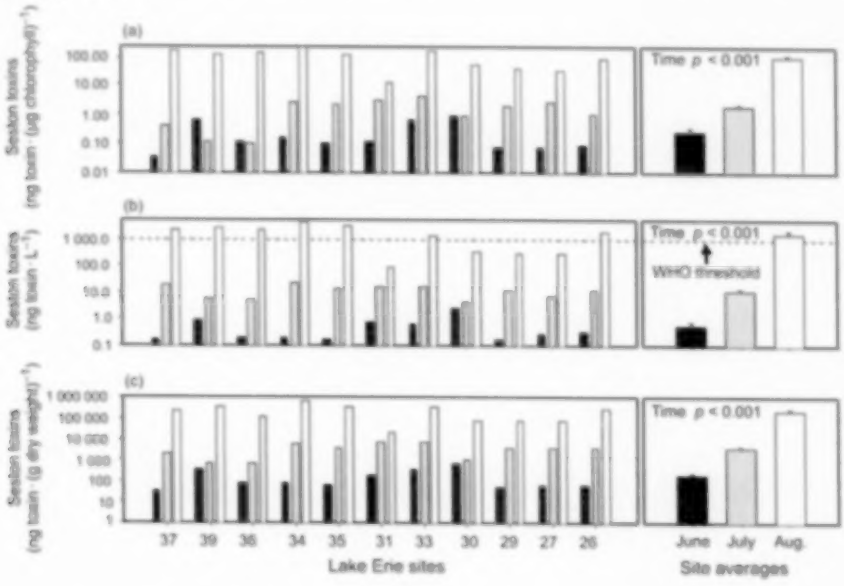
Fig. 2. Mean seston microcystin concentrations measured as microcystin-LR equivalents for integrated water samples collected from the surface to near the lake bottom at 11 sampling stations during the summer of 2006: (*a*) ng toxin·(μg chlorophyll)$^{-1}$; (*b*) ng toxin·L^{-1}; (*c*) ng toxin·(g dry weight)$^{-1}$. Left panels contain monthly site concentrations (n = 1 per site). Right panels contain monthly averages (± standard error, SE) across sites (n = 11 per date). Sampling dates: solid bars, 22 June 2006; shaded bars, 18 July 2006; open bars, 22 August 2006. Note the $\log_{10}$ scale of the y axes.



tions (measured as ng toxin·(μg chlorophyll)$^{-1}$, ng toxin·L^{-1}, and ng toxin·(g dry weight)$^{-1}$) showed similar patterns to algal abundance (ANOVA, time p < 0.001; Figs. 2*a*, 2*b*, 2*c*), and consequently both variables were positively correlated ($r \geq 0.440$, $p \leq 0.010$, n = 33). For all sampling sites and dates, microcystin concentrations in the seston were in the range of 0.04–189 ng toxin·(μg chlorophyll)$^{-1}$, 0.16–4284 ng toxin·L^{-1}, and 34 – 767 760 ng toxin·(g dry weight)$^{-1}$. In August, concentrations of microcystins exceeded the level (1 μg toxin·L^{-1}; Chorus and Bartram 1999) set by the WHO for safe drinking water standards (mean, 1.68 μg toxin·L^{-1}; range, 0.08–4.28 μg toxin·L^{-1}), but Lake Erie microcystin concentrations were below this recommended limit in June and July (range, 0.0002–0.02 μg toxin·L^{-1}; Fig. 2*b*).

Although mean (± standard error, SE) length and weight of analyzed yellow perch increased during the year (June, 166 ± 6 mm, 63 ± 5 g, n = 22; July, 174 ± 5 mm, 70 ± 5 g, n = 24; August, 184 ± 5 mm, 81 ± 6 g, n = 22), these seasonal trends were not statistically significant (ANOVA, $p \geq 0.069$). Further, total gut contents (grams dry mass of gut contents per gram dry mass of fish) did not vary over time (ANOVA, p = 0.076) for yellow perch collected in June (mean ± 1 SE = 0.127 ± 0.024 g), July (0.318 ± 0.108 g), and August (0.126 ± 0.048 g). Of the prey that could be identified, relative abundances (by dry weight) of the three prey types varied over time but were not correlated to yellow perch muscle or liver toxin concentrations ($p \geq 0.054$). Benthic invertebrates were relatively more abundant in yellow perch guts during June (mean ± 1 SE = 76% ± 11%, n = 16) than in July (19% ± 9%, n = 19) and August (44% ± 14%, n = 12; ANOVA, p = 0.001). The abundance of fish in yellow perch guts increased over time (mean ± 1 SE; June, 18% ± 10%, n = 16; July, 46% ± 11%, n = 19; August, 48% ± 14%, n = 12; Kruskal–Wallis, p = 0.045). Although no identifiable zooplankton were observed in yellow perch guts collected during August (mean ± 1 SE = 0% ± 0%, n = 12), zooplankton were significantly less abundant in yellow perch collected in June (mean ± 1 SE = 7% ± 6%, n = 16) than in July (mean ± 1 SE = 36% ± 10%, n = 19; ANOVA, p = 0.010).

Liver microcystin concentrations in individual yellow perch ranged from 17 to 1182 ng toxin·(g dry weight)$^{-1}$ and varied significantly across sites in July (ANOVA, p = 0.033; Fig. 3*a*), but not in June or August (ANOVA, $p \geq 0.086$; Fig. 3*a*). In general, fish collected closer to Maumee Bay appeared to have lower liver microcystin concentrations than those from sites in the central area of the western basin of Lake Erie (Figs. 1 and 3). Moreover, we found no differences in yellow perch liver toxin concentrations over time (ANOVA, p = 0.372; Fig. 3*a*). Liver microcystin concentrations were unrelated to fish length or wet weight (all fish data: length, r = 0.215, p = 0.078, n = 68; weight, r = 0.203, p = 0.097, n = 68). Similarly, mean monthly yellow perch liver toxin concentrations averaged within each site were not significantly correlated with seston microcystin concentrations, measured as microcystins per volume, dry weight, or chlorophyll content, throughout the summer of 2006 (site- and time-averaged data: $r \leq -0.107$, $p \geq 0.609$, n = 25).

Microcystins concentrations in individual yellow perch muscle tissues ranged from 0.12 to 4.02 ng toxin·(g dry weight)$^{-1}$ and significantly varied across sites in August (ANOVA, p < 0.001; Fig. 3*b*), but not in June or July

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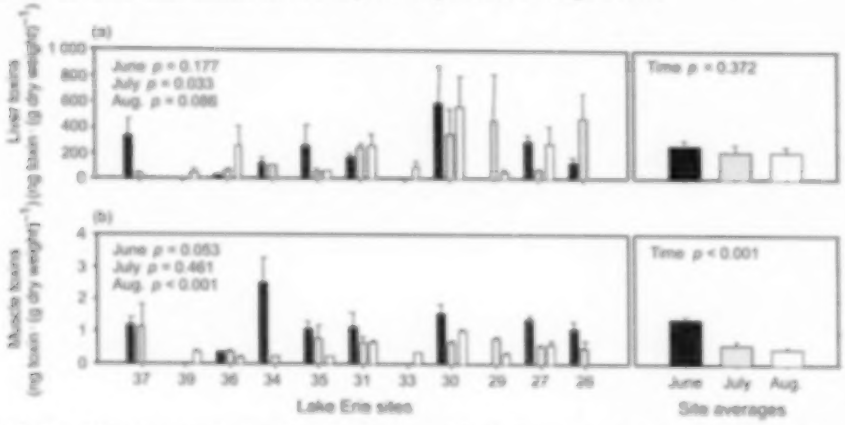
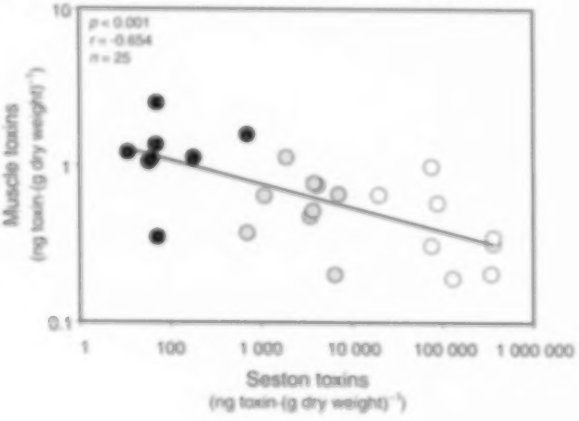
Fig. 3. Mean (± standard error, SE) (*a*) liver and (*b*) muscle microcystin concentrations measured as microcystin-LR equivalents (ng toxin·(g dry weight)$^{-1}$) for yellow perch collected at 11 sampling stations during the summer of 2006. Left panels contain monthly site averages (n = 0–3 per site). Right panels contain monthly averages across sites (n = 22 (for June and August) or 24 (for July) per date). Sampling dates: solid bars, 22 June 2006; shaded bars, 18 July 2006; open bars, 22 August 2006.



(ANOVA, $p \geq 0.053$; Fig. 3*b*). In general, muscle microcystin concentrations of yellow perch were relatively low, and muscle toxin concentrations, measured as ng toxin·(g dry weight)$^{-1}$, represented only 0.8% (SE = 0.1%, n = 68) of the toxin found in liver tissue. However, we found large differences in yellow perch muscle toxin concentrations over time across all sites (ANOVA, $p < 0.001$; Fig. 3*b*). In contrast to the pattern observed for seston toxin concentration, which increased over time, muscle microcystin concentrations decreased over time (Figs. 3 and 4). Individual yellow perch muscle toxin concentrations were negatively related to total lengths, wet weights, and liver toxin concentrations (all fish data: length, r = –0.282, p = 0.020, n = 68; weight, r = –0.302, p = 0.012, n = 68; liver concentrations, r = 0.361, p = 0.003, n = 68). Interestingly, across sites from June to August 2006, mean yellow perch muscle microcystin concentrations were negatively related to seston microcystin concentrations regardless of whether seston toxins were measured relative to chlorophyll abundance, weight of material extracted, or volume of water filtered (site- and time-averaged data: $r \geq 0.6039$, $p \leq 0.001$, n = 25; Fig. 4).

Fig. 4. Correlation between seston microcystin concentration (ng toxin·(g dry weight)$^{-1}$) and mean yellow perch (*Perca flavescens*) muscle microcystin concentration (ng toxin·(g dry weight)$^{-1}$) for all 11 sampling stations and three sampling dates (solid circles, 22 June 2006; shaded circles, 18 July 2006; open circles: 22 August 2006). Note that all data are represented as microcystin-LR equivalents. Each data point represents an n = 1 for seston toxins and an n = 1–3 for muscle toxins. Note the $\log_{10}$ scale of both axes.



Discussion

During July and August of 2006, the western basin of Lake Erie experienced a high biomass of phytoplankton (maximum site-specific algal abundance of 47 µg chlorophyll·L^{-1}) containing the toxigenic cyanobacterium *Microcystis aeruginosa*. Seston microcystin levels increased throughout the summer and exceeded the threshold established by the World Health Organization for safe drinking water (1 µg microcystin·L^{-1}; Chorus and Bartram 1999) at 7 of the 11 sites sampled in August 2006. Although orders of magnitude lower than seston toxin levels (maximum seston concentration of 768 µg toxin·(g dry weight)$^{-1}$), yellow perch liver samples contained relatively high amounts of microcystin (maximum liver concentration of 1.2 µg toxin·(g dry weight)$^{-1}$). In contrast, yellow perch muscle toxin concentrations remained relatively low throughout the summer (maximum muscle concentration of 0.004 µg toxin·(g dry weight)$^{-1}$). Further, although there was no clear relationship between yellow perch liver toxin levels and seston toxin concentrations over time, yellow perch muscle microcystin levels were negatively related to seston toxin concentrations.

Given the significance of protecting freshwater drinking and recreational water supplies, it is important to explore, across aquatic systems, toxic intracellular cyanobacterial compounds, such as microcystin (Chorus and Bartram 1999; Zurawell et al. 2005). Surprisingly, few data exist for measured seston toxin levels in the Laurentian Great Lakes (Babcock-Jackson 2000; Murphy et al. 2003; Dyble et al. 2008) and its watershed (Watzin et al. 2006; Lehman 2007; Knoll et al. 2008). Instead, research has been directed at

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quantifying microbial diversity and the potential for cyanobacterial toxin production (e.g., *mcy* gene analysis) in waterbodies within the region (Wilson et al. 2005; Ouellette et al. 2006; Rinta-Kanto and Wilhelm 2006). Available toxin data for the Great Lakes show large spatial (western basin of Lake Erie: range = 0.1–15.4 μg toxin·L^{-1}; Rinta-Kanto et al. 2005) and temporal (western basin of Lake Erie: range = 0.002–0.085 μg toxin·L^{-1} (Babcock-Jackson 2000); Hamilton Harbor, Lake Ontario: range = 0–239 μg toxin·L^{-1}; eastern basin of Lake Erie: range = 0–0.4 μg toxin·L^{-1} (Murphy et al. 2003)) variation in summer seston microcystin concentrations, which is consistent with our observations in western Lake Erie (range = 0.0002–4.3 μg toxin·L^{-1}). Further, we found expected patterns in seston toxins within and across sites, with higher seston toxin concentrations later in the summer and at sites near mesotrophic Maumee Bay, respectively.

Field sampling techniques can significantly influence toxin estimates (Tillmanns et al. 2007), and our seston toxin data should be viewed as conservative, as the samples were integrated water samples (i.e., surface to near bottom). In contrast, other studies have measured seston toxin levels collected in the top 1 m of the water column (Murphy et al. 2003; Rinta-Kanto et al. 2005). If we had sampled the surface water, our seston toxin data likely would have been much higher on calm days when thick scums of buoyant cyanobacteria coated the surface of Lake Erie. Although surface samples are useful for estimating toxin concentrations that pose a threat to livestock and human health through tainted drinking water (Chorus and Bartram 1999), integrated samples were collected to estimate toxin concentrations throughout the water column (in part because yellow perch are rarely found near the lake surface). Moreover, few studies provide toxin data in units (e.g., toxin per biomass) that can be easily compared across trophic levels (but see Murphy et al. (2003) and Ibelings et al. (2005)). Instead, most studies present seston toxin data as μg toxin·L^{-1}, which is the unit typically considered by the World Health Organization (Chorus and Bartram 1999). Toxin per biomass data are easily collected by drying samples prior to weighing and are useful for direct comparisons among trophic levels both within and across studies. Given that water content of phytoplankton, zooplankton, fishes, bivalves, and other aquatic biota will vary, we suggest that future similar studies provide toxin data on a per dry biomass basis (ng toxin·(g dry weight)$^{-1}$).

Besides posing serious threats to global freshwater supplies, cyanobacterial toxins contained within fish tissues may present an alternative route of exposure to humans. However, this mechanism is poorly understood given that relatively few studies have determined the concentration of cyanobacterial toxins in the muscle tissue of fishes typically consumed by humans (Magalhães et al. 2001; Xie et al. 2004; Wood et al. 2006). Instead, many studies have documented toxin levels in those tissues where cyanobacterial toxins aggregate, e.g., liver tissue for the hepatotoxic microcystin (Kotak et al. 1996; Zimba et al. 2001; Ibelings et al. 2005). Moreover, in contrast to some past studies that analyzed the tissues of one or a few individuals for multiple fish species (Murphy et al. 2003; Gkelis et al. 2006), our focused study on the muscle and liver tissues from 68 yellow perch collected in the western basin of Lake Erie allowed us to draw more significant conclusions for this commercially important fish.

For the Great Lakes region, few data exist for microcystin levels in fish muscle or liver tissues (Babcock-Jackson 2000; Murphy et al. 2003). To our knowledge, the only other available toxin data for Lake Erie yellow perch are presented by Babcock-Jackson (2000), who showed liver microcystin concentrations of young-of-the-year (range of 94–230 ng toxin·(g wet weight)$^{-1}$, n = 10–15) and adult (127 ng toxin·(g wet weight)$^{-1}$, n = 7) yellow perch collected during 1998. Murphy et al. (2003) reported low toxin concentrations in the muscle (1 ng toxin·(g wet weight)$^{-1}$) and liver (1 μg toxin·(g wet weight)$^{-1}$) tissues of round gobies collected in Hamilton Harbor, Lake Ontario, during the summer of 2001. If the dry weight of fish tissue is 20% of the wet weight, data from these two studies for yellow perch and round gobies correspond to toxin levels of 5 ng toxin·(g dry weight)$^{-1}$ for muscle tissue and 5–1150 ng toxin·(g dry weight)$^{-1}$ (range) for liver tissue, which is consistent with our findings of toxin concentrations in yellow perch muscle (range of 0.12–4.02 ng toxin·(g dry weight)$^{-1}$) and liver tissue (range of 17–1182 ng toxin·(g dry weight)$^{-1}$).

Intriguingly, we found a significant, negative correlation between average seston and yellow perch muscle microcystin concentrations ($p < 0.001$), i.e., when seston toxins were lowest, muscle toxins were highest. Few studies have simultaneously measured microcystin abundances in the seston and fish muscle tissue collected from a lake over time. Of those, Magalhães et al. (2001) provided a similar negative relationship between seston and muscle microcystin concentrations for at least one year of their study. The other studies (Magalhães et al. 2003; Chen et al. 2006) showed a positive relationship between these variables. We consider finding these types of relationships between seston and fish muscle tissue microcystin concentrations within a lake to be remarkable given that the water mass where the fishes were found and that where they were exposed to microcystins are likely different because of the movement of the fishes, zooplankton prey, or the water through circulation within a lake. There is no single obvious explanation for this pattern as it is assumed that perch acquire cyanobacterial toxins through their diet, including zooplankton that prey on phytoplankton, such as toxigenic cyanobacteria. One potential explanation is that early in the summer when cyanobacteria have not become dominant, zooplankton populations are growing well while grazing on mixed diets containing various types of bacteria, cyanobacteria, and phytoplankton. When large-bodied zooplankton are abundant, yellow perch may preferentially consume such prey (which may have accumulated microcystins through their diet; Thostrup and Christoffersen 1999; Smith and Haney 2006). However, because of lower food quality (i.e., increasing dominance by cyanobacteria) and an increase in fish predation, large zooplankton are typically progressively replaced by smaller zooplankton (Brooks and Dodson 1965) during the seasonal succession of the plankton community towards cyanobacterial dominance, making zooplankton less important for yellow perch sustenance and thus reducing yellow perch exposure to microcystins. Yellow perch diet data support this idea, given that we found variation in the relative abun-

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dances of zooplankton in the perch diets for fishes collected in June, July, and August (ANOVA, $p = 0.010$) and, in fact, found no identifiable zooplankton in the guts of yellow perch collected in August. Further, this seasonal pattern is consistent with that of previous studies (Tyson and Knight 2001).

Another explanation for the negative relationship between seston and perch muscle toxins could be related to the sizes of perch analyzed. Although fish size (measured as total length and wet weight) did not show significant trends, we did analyze slightly (insignificantly) smaller fish in June compared with in July and August. As fish grow, they tend to shift their diets from primarily small-bodied prey (e.g., zooplankton) to primarily large-bodied prey (e.g., benthic macroinvertebrates and fish). However, a multiple regression analysis describing mean muscle toxin levels as a function of mean seston toxins and perch wet weight estimates for each site per month ($n = 25$) reconfirmed that yellow perch size does not account for a significant amount of variation in muscle microcystin concentrations (dependent variable = log muscle toxins; independent variables = log seston toxins, $p < 0.001$, log wet weight, $p = 0.331$). Finally, because yellow perch feed on benthic organisms and many cyanobacteria overwinter on the sediments (Reynolds et al. 1981), it is possible that the counterintuitive pattern between seston and muscle toxins is related to toxin accumulation or biomagnification through the benthic food web. However, this mechanism for trophic transfer of cyanobacterial toxins is poorly understood. Thus, we support future observational and experimental studies intended to more fully understand the trophic transfer of cyanobacterial toxins.

Another motivation of this study, besides determining if microcystins are present in yellow perch tissues, was to determine if this class of hepatotoxins found within the edible muscle tissue of yellow perch could be harmful to human consumers. To determine this risk, we calculated a conservative microcystin total daily intake (TDI) threshold, which is the acceptable amount of a toxic compound that an individual can consume per day for a lifetime, based on established WHO (0.04 μg toxin·(kg of body weight)$^{-1}$·day^{-1}; Chorus and Bartram 1999) and proposed US Environmental Protection Agency (0.003 μg toxin·(kg of body weight)$^{-1}$·day^{-1}; US EPA 2006) guidelines. For a human weighing 80 kg and consuming 25.4 g dry weight (wet to dry weight conversion of 5:1) of fish tissue per day (Keill and Kissinger 1999, based on a consumption estimate from 90% of a Native American population that relies on a fish diet), the maximum concentration of muscle toxins we observed in yellow perch (maximum of 4.02 ng toxin·(g dry weight)$^{-1}$) was well below thresholds estimated using established WHO (117 ng toxin·(g dry weight)$^{-1}$) and proposed EPA (9.5 ng toxin·(g dry weight)$^{-1}$) guidelines.

Our findings show that even during an intense, large-scale bloom of microcystin-producing *Microcystis aeruginosa* in which seston toxin levels exceeded the threshold established by the WHO for microcystin in drinking water (Chorus and Bartram 1999), muscle tissue from the sportfish yellow perch contained concentrations of microcystin well below conservative thresholds established for human consumption. However, it is important to note that humans consuming fish tissue can potentially be exposed to both free and bound forms of toxins. Within organisms, microcystin may exist primarily in bound form owing to biotransformation. In fact, the bound form was found to represent 76% of the microcystin observed in salmon (*Salmo salar*) liver (Williams et al. 1997). Thus, total microcystin concentrations in yellow perch tissues may be higher than reported herein, given our inability to quantify covalently bound microcystins because of the limitations of our extraction technique. However, it is well recognized in toxicology that bound or conjugated forms of compounds are not readily absorbed, as these forms are created through biotransformation for detoxification and elimination of toxic compounds. Despite this general understanding, no studies adequately elucidate the actual potential for absorption of such forms of microcystin by humans. The toxicity of some metabolites of microcystin have been examined in mice using intravenous injection, and the glutathione and cysteine conjugates were found to be about 10 times less toxic than the parent microcystin-LR (Kondo et al. 1992). Thus, evaluation of the potential human exposure to cyanobacterial secondary metabolites would be best served by monitoring the extractable parent microcystin (because of limited bioaccumulation potential and toxicity of conjugates).

Moreover, understanding whether the low microcystin levels that we observed in the edible muscle tissues of Lake Erie yellow perch during a cyanobacterial bloom are typical will depend on many abiotic and biotic parameters. For example, populations of cyanobacteria exhibit high genetic and physiological diversity (Janse et al. 2004; Via-Ordorika et al. 2004; Wilson et al. 2006*b*), which could influence toxin dynamics. Moreover, individual strains of cyanobacteria have been shown to significantly alter their growth rates and toxin production in response to changes in temperature, light level, and availability of nutrients (Lee et al. 2000; Oh et al. 2000; Wiedner et al. 2003). In addition, some zooplankton may adapt to graze on toxic cyanobacteria (Hairston et al. 2001; Sarnelle and Wilson 2005), whereas some benthic grazers can actively avoid consuming cyanobacteria (Vanderploeg et al. 2001). Such ecological interactions may strongly influence the flow of cyanotoxins through food webs, although this has yet to be determined empirically. Thus, it is unclear if the trophic transfer of cyanobacterial toxins will always culminate in low fish muscle toxin concentrations. Additionally, although our results suggest that humans are not in danger of cyanotoxin poisoning when consuming muscle tissues from yellow perch harvested during cyanobacterial blooms, toxin concentrations in yellow perch livers exceeded conservative consumption thresholds for microcystin. Piscivorous fishes that feed on yellow perch could accumulate potentially dangerous levels of microcystins and could pose a health risk to humans who consume these fishes, but this possibility has not been well studied. Thus, there may be a health threat to humans consuming whole fish or fish livers during cyanobacterial blooms, and this threat should be communicated to public health officials. Consequently, we encourage future studies to quantify the type and abundance of toxic intracellular cyanobacterial compounds in important commercially and recreationally harvested fish species.

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Sandeel (*Ammodytes marinus*) larval transport patterns in the North Sea from an individual-based hydrodynamic egg and larval model

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Abstract: We have calculated a time series of larval transport indices for the central and southern North Sea covering 1970–2004, using a combined three-dimensional hydrodynamic and individual-based modelling framework for studying sandeel (*Ammodytes marinus*) eggs, larval transport, and growth. The egg phase is modelled by a stochastic, nonlinear degree-day model describing the extended hatch period. The larval growth model is parameterized by individually backtracking the local physical environment of larval survivors from their catch location and catch time. Using a detailed map of sandeel habitats in the North Sea, the importance of hydrography for early life stages of sandeel to their recruitment success is explored. We find that the sandeel larval transport patterns in the North Sea are relatively robust toward uncertainties in biological parameters, when mortality aspects are included. We find only weak spatiotemporal correlations between elements of the transport indices in the time series, mainly positive correlation between retention terms for the same year. The transport connectivity of sandeel habitats in the North Sea and the dynamical properties of the North Sea transport system are also analyzed, and we introduce novel a scheme to quantify direct and indirect connectivity on equal footings in terms of an interbank transit time scale.

Résumé : Nous avons calculé une série chronologique d'indices de transport des larves pour les régions centrale et australe de la mer du Nord pour la période 1970–2004 à l'aide d'un cadre de modélisation hydrodynamique tridimensionnel et basé sur l'individu, afin d'étudier le transport et la croissance des oeufs et des larves du lançon (*Ammodytes marinus*). La phase des oeufs peut se représenter par un modèle stochastique non linéaire de degrés-jours qui décrit la période prolongée de l'éclosion. Les paramètres du modèle de croissance larvaire s'obtiennent en retraçant l'environnement physique local individuel des larves survivantes à partir du lieu et du moment de leur capture. Une carte détaillée des habitats des lançons dans la mer du Nord permet d'explorer l'importance de l'hydrographie chez les premiers stades de vie des lançons pour le succès du recrutement. Nous observons que les patrons de transport des larves de lançons dans la mer du Nord sont relativement robustes vis-à-vis les incertitudes des variables biologiques lorsqu'on tient compte des aspects de la mortalité. Il n'existe que de faibles corrélations sputio-temporelles entre les éléments des indices de transport dans la série chronologique, principalement des corrélations positives entre les différents termes de la rétention dans une même année. Nous analysons aussi la connectivité des habitats par le transport de lançons dans la mer du Nord et les propriétés dynamiques du système de transport de la mer du Nord; nous présentons une méthode inédite pour calculer les connectivités directe et indirecte sur une base égale sur l'échelle du temps de passage d'un banc à un autre.

[Traduit par la Rédaction]

Introduction

The lesser sandeel (*Ammodytes marinus*) plays a pivotal role as a midtrophic wasp-waist component in the North Sea ecosystem and is the basis for an important fishery. After metamorphosis from larvae to juveniles, the sandeels settle, i.e., they bury in the sediment during times when they do not feed on zooplankton (see, e.g., Winslade 1971). Sandeels are assumed to remain resident on sandbank habitats (Kunzlik et al. 1986). North Sea lesser sandeels spawn in midwinter and the eggs stick to the sand grains at the seabed until hatch (Macer 1966). The relatively short larval drift phase of 1–3 months (Wright 1993; Wright and Bailey 1996; Jensen 2001) in February to May is believed to render the sandeel recruitment rather sensitive to optimal hydrographic conditions (Wright and Bailey 1996).

Present sandeel stock-assessment models treat the North Sea sandeel populations as one single homogeneous stock or, at most, two stocks. These stock-assessment models neither predict stock variations sufficiently well nor provide a

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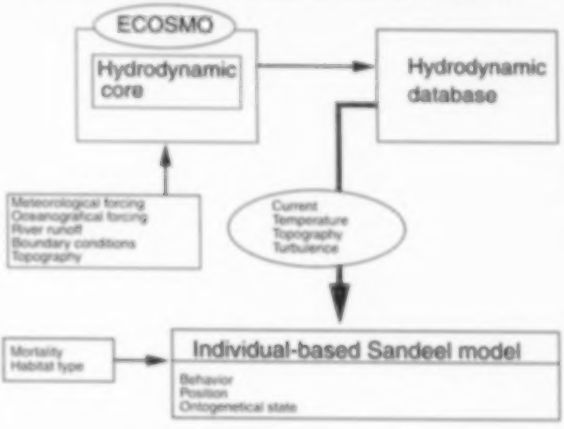
satisfactory starting point for linking population dynamics to underlying oceanographic and biological processes that are major drivers for the population dynamics of many fish species including sandeels (Arnott and Ruxton 2002). This detailed understanding appears highly important presently, when ecosystems face potential regime shifts triggered either directly by global warming or by cascade effects of global warming. Statistical models inferred from hindsight time series are uncertain when the dynamic regime of the ecosystems changes, because their forecasts are based on the past dynamics in a different regime. Alternative and potentially more powerful tools are based on deterministic modelling of underlying processes using invariant laws of nature.

Our working hypothesis is that spatially explicit population dynamics is necessary to improve the description of the stock–recruitment relationship for sandeels and that adult sandeel population survival and growth is regulated locally by spatially varying mortality and zooplankton availability. However, spatiality per se may trigger new effects not accessible in models without spatial freedom, like coexistence, patchiness, or fluctuations in the population distribution. On the biological side, we also test hypotheses about the importance of variable, extended hatch periods (compared with a fixed day) and hypotheses about the mechanism controlling the juvenile settlement process (a flexible settlement schedule compared with a fixed body length trigger), and finally, we test hypotheses about the impact of exogene and endogene factors on larval growth.

Earlier hydrodynamic studies of sandeel larval transport already have stressed the importance of advective transport. Berntsen and co-workers (Berntsen et al. 1994) considered a long time series for passive advection in the North Sea but had no integrated larval growth model and lacked other key biological aspects controlling the physical–biological interaction for sandeel larvae. They found that retention was larger in the southern parts of the North Sea than in the northern parts. Other more recent studies (Proctor et al. 1998; Gallego et al. 2004) have focused on a limited region of the North Sea shelf, the Orkney–Shetland Isles and the Scottish Isles. Using a barotrophic hydrodynamic model on a coarse grid, Proctor et al. (1998) suggested that larval advection from Orkney to Shetland was important for Shetland recruitment in some years. Both studies tried to juxtapose virtual population analysis (VPA) recruitment data with transport indices, but both studies obtained only a moderate explanation of recruitment variability. In the present study, we will argue that a moderate correlation between recruitment and transport indices alone is what should be expected. With focus on closed-area effectiveness, Gallego et al. (2004) also suggested that some southern migration was possible between the Scottish Isles, but no comparison with recruitment observations were attempted.

In the present study, we provide an updated panoptic quantification of the central and southern North Sea transport patterns over a 35-year period using state-of-art three-dimensional (3D) hydrodynamic and individual-based modelling and show how to include transport indices into a coherent, process-oriented recruitment model framework.

Fig. 1. Sketch of coupled hydrodynamic and individual-based model (IBM) model SLAM (sandeel larval advection model).



Materials and methods

Based on the coupled ecosystem model framework ECOSMO (Schrum et al. 2003, 2006; Hochbaum 2004), a coupled hydrodynamic and individual-based model (IBM) has been developed for the early life stages (egg–larvae) of the lesser sandeel (Fig. 1). This model is called the sandeel larval advection model (SLAM).

Hydrodynamic model

The hydrodynamic module of ECOSMO (Schrum and Backhaus 1999; Schrum et al. 2006) is a free-surface, z-level model formulated on a staggered Arakawa C-grid. The model uses an approximate horizontal resolution of 5 nm and 5 m layers from the surface down to a depth of 40 m (and 8 m layers below 40 m depth). The model uses a terrain following lowest layer with spatially varying thickness. The Eulerian grid scale vertical turbulent diffusivity ($K(x,t)$) is used to describe larval dispersal in the IBM. Turbulent processes in ECOSMO are parameterized using an analytical k–ε approach, considering counteracting effects of local sheer and stratification (Schrum 1997). The ECOSMO hydrodynamic model setup has been successfully validated against available observations (Janssen et al. 2001; Janssen 2002), particularly with respect to its performance in describing the interannual variability of hydrodynamic conditions. The detailed validations illustrated the model's ability to describe successfully seasonal and intraannual variability of hydrodynamic processes in the North Sea, hence justifying its employment in the study of hydrodynamic impacts on the North Sea ecosystem. For the present study, we used hydrodynamic model results from a previously performed long-term simulation from 1958–2004 (Schrum et al. 2003). The hydrodynamic fields from the ECOSMO model (currents, temperature, salinity, turbulence) are applied to the IBM as daily-averaged fields (for data-compression purposes).

Individual-based biological models

The biological states are updated daily, based on the physical environment. The biological part of the IBM con-

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tains egg and larvae submodels. In the following sections, we will detail the submodels of the various aspects of sandeel early life history used in the present study. To label alternative composite larval models in a transparent way, we use the following designation template:

(1) Model = initialization_settlement

Each considered aspect (initialization (e or h), settling (S40 or flx)) is associated with a model identifier (a tag) that corresponds to the actual submodel employed in the simulation; below we will elaborate the submodel alternatives in eq. 1. For instance, the reference model used below is labelled h_S40.

Initialization of larval–egg populations

We model the hatch process stochastically. The extended hatch period is hypothesized to be an important part of the sandeel life strategy, because larval recruitment success is believed to be strongly dependent on proper timing with secondary marine production (Wright and Bailey 1996). Strictly deterministic modelling of the hatch process will give a time-peaked hatch process if eggs are spawned at a specific time. This would not be in correspondence with field observations (Jensen 2001) or well-controlled laboratory experiments (Smigielski et al. 1984).

In our setup, tracers may be initialized in an arbitrary egg or larval state (or a mixed set). Eggs are immobile and demersal, whereas larvae are pelagic (but released at the bottom at hatch time). Eggs and larvae are initially positioned at suitable spawning grounds in the North Sea, as described below. Spawning grounds are assumed coincident with sandeel habitats, as no spawning migration has been observed. In this work, we will consider two opposite schemes for larval release to explore the influence of hatch period length on transport: first by releasing larvae directly on a single day (20 February) with an initial length L_0 at the sea bed; this initialization model is designated h in eq. 1. Secondly, we let the hatch time distribution be determined by an egg development model (giving an extended hatch period), as described below. Eggs are spawned demersally at a constant rate at 1 January; this initialization model is designated e in eq. 1.

The physically released larvae population is mathematically sampled by a set of representative tracers, each representing a fixed number of individuals (the ratio of real physical larvae per tracer need not be stipulated, as density effects are not addressed explicitly, only relative numbers matter).

Stochastic egg development model and hatching

In model e, the eggs are developed according to a hatch probability function, as parameterized from the egg maturation experiment of Smigielski et al. (1984) (Fig. 2*b*). The lines in Fig. 2 show smooth temperature fits to the statistical delimiters of the hatch process: beginning, median, and end, as function of temperature (T, in degrees Celsius):

(2) $$F(T) = Ae^{-kT}$$

where $F(T)$ is either hatch beginning ($b(T)$), hatch median ($m(T)$), or hatch end ($e(T)$). Parameters for hatch process marker functions are found by standard nonlinear regression (Table 1).

Table 1. Parameters for stochastic egg development model (model e) at hatch beginning ($b(T)$), hatch median ($m(T)$), or hatch end ($e(T)$).

	$b(T)$	$m(T)$	$e(T)$
A (days)	78.484	108.75	176.14
k (C^{-1})	0.10984	0.12488	0.11358

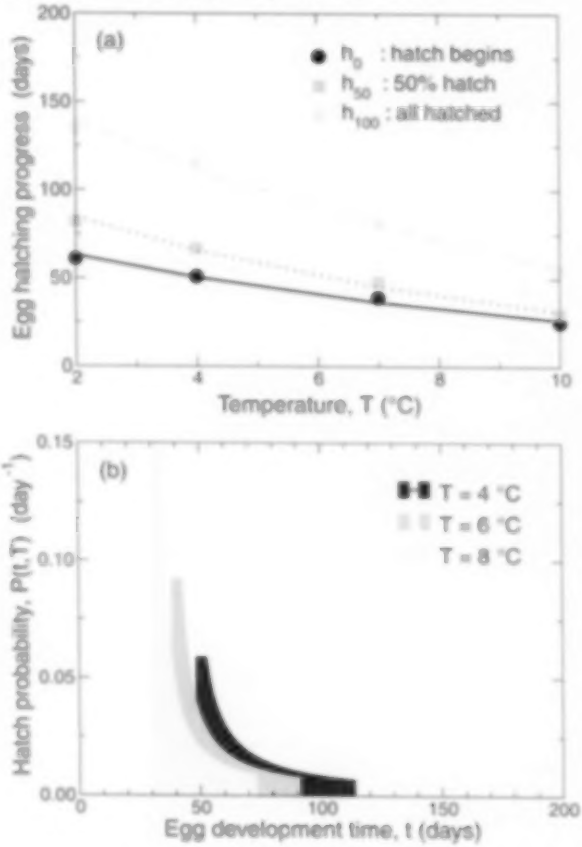

Fig. 2. Stochastic egg development model based on Smigielski et al. (1984). (*a*) When moving up vertically at a given temperature, the curves show the observed egg hatch progress (symbols are observation data points, lines are curve fits). (*b*) Continuous clutch hatch frequencies, corresponding to curves in (*a*).

These fits are mapped to an isothermal hatch probability function that depends parametrically on temperature (Fig. 2*a*):

(3) $$p_T(t) = \begin{cases} 0 & t < b(T) \\ \dfrac{1}{n(T)}\dfrac{1}{t - g(T)} & b(T) < t < e(T) \\ 0 & t > e(T) \end{cases}$$

where t is day after spawning. Each egg is assigned a fixed relative growth rate, which reflects the idea that an embryo

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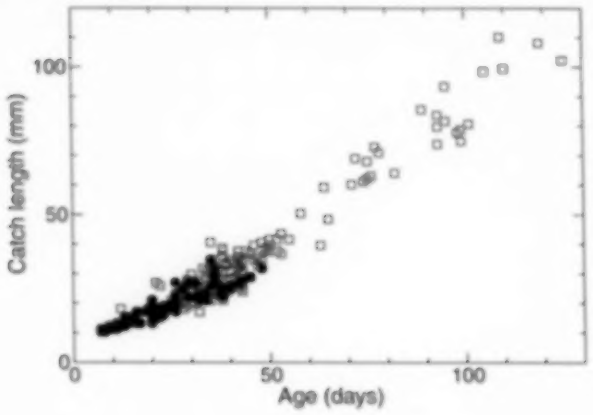
Fig. 3. Age–length data for 1995 (solid circles) and 1996 (open squares) of pre- and post-metamorphosed larvae.



develops according to its genetic composition, as well as local physical parameters, like the oxygen tension (see, e.g., review by Kamler 2002), and local chemical transport coefficients. The relative magnitudes of these properties between eggs are strongly dependent on physical location of the individual egg; therefore the relative growth rate among eggs in the same cluster is assumed to be maintained, irrespective of fluctuations in the local physical environment. Technically, this corresponds to a stochastic nonlinear degree-day model. A deterministic linear degree-day model will display as a single hyperbola in Fig. 2*a*, or a hyperbolic envelope for a stochastic linear degree-day model. An ensemble of such eggs described by the development eq. 3 above will reproduce the hatch distributions in Fig. 2*a*, at a given temperature. In Appendix A, we give expressions for the auxiliary functions $n(T)$ and $g(T)$. The choice of $p_T(t)$ is the simplest asymmetric distribution that produces deviates directly from a mapping of a uniform distribution. We do not feel that the currently available hatch process data for the sandeel species warrants a more sophisticated parameterization. When eggs are hatched according to the stochastic egg model, each egg is automatically promoted to a larval individual starting at the seabed.

Larval growth models and data

The larval growth models accounts for the length growth of larvae as a deterministic, daily increment, according to

$$(4) \qquad \frac{dL}{dt}(L,T) = \lambda(T)\left(\frac{L}{L_0}\right)^{\gamma}\left(1-\frac{L}{L_\infty}\right)$$

The motivation for this is as follows. In the first term, temperature (T) acts as a proxy for bioenergetics effects (zooplankton production rate, metabolism, and activity level). In the second term, the larval length (L) accounts for the dominating size scaling effect in sandeel larval growth, which encompasses ontogenetic effects. The last term express growth saturation for $L \to L_\infty = 218$ mm (Macer 1966), due to increasing resting metabolism and reproduction investment. The effect of the last term becomes noticeable only after the size of metamorphosis, $L = L_m \sim 40$ mm (Wright and Bailey 1996; Jensen 2001). No memory effects in daily growth (like larval condition) are included. The temperature modulation ($\lambda(T)$) is parameterized by a nonnegative factor as

Table 2. Growth parameters for larval growth (in units of mm·day^{-1}).

Model	γ	λ_0	λ_1 (1/°C)	Variance explanation (%)
1	0*	–0.642	0*	88.0
2	0*	–1.441	0.123	89.7
3	0.316	–1.725	0.136	90.1

Note: An asterisk (*) means that the parameter has been fixed to that value at model parametrization. Growth model 3 is the reference model used in simulations in this paper, unless otherwise stated.

$$(5) \qquad \lambda(T) = e^{\lambda_0+\lambda_1 T}$$

This simple growth model is parameterized from North Sea sandeel samples by MIK trawl and bottom dredge from 1995 and 1996 (Jensen 2001). Larval ages were obtained by individual otolith analysis (Wright 1993). The 1995 sample (80 larvae from seven locations) covers mostly premetamorphosed larvae, whereas the 1996 sample (124 sandeels from eight locations) covers mostly metamorphosed juveniles and settled sandeels. The spatial coverage of larval sampling stations in both years is frontal areas in the central and eastern North Sea. The larval age–length data from 1995 and 1996 plotted together appear to follow a common functional relation (Fig. 3).

Although sandeel larvae develop active swimming abilities before they metamorphose at a length $L_m \sim 40$ mm (Wright and Bailey 1996; Jensen 2001), we have assumed that they generally follow currents as passive tracers until they settle at the point of metamorphosis. The sensitivity of L_m is tested later.

Premetamorphosis larval growth models

To parameterize the growth model (eqs. 4 and 5) above, we reconstruct the temperature history ($T_i(t)$) of individual larvae using the hydrodynamic backtracking scheme described recently (Christensen et al. 2007). Integrating eq. 4 above for each larva, we obtain a predicted length:

$$(6) \qquad \bar{L}_i = H(T_i(t_i^b < t < t_i^c), t_i^c, t_i^b; \gamma, \lambda_0, \lambda_1)$$

where t_i^c denotes catch day of larva i, and t_i^b is the hatch day (inferred from otolith reading). Notice that the first argument of $H(\bullet)$ above is the entire temperature history, not just a single temperature. By minimizing the sum of residuals between predicted larval lengths and observed larval lengths, we estimate growth parameters ($\gamma, \lambda_0, \lambda_1$) as a hierarchy of increasing complexity (Table 2). Model 1 (the null model) expresses constant growth, model 2 includes a temperature modulation term $\lambda(T)$, and model 3 additionally contains the allometric term with $\gamma \neq 0$. $L_0 = 7.73$ mm was inferred by extrapolation to hatch day in the figure using a quartic polynomial (this is similar to the largest size yolk sac larvae in samples from the western North Sea (Wright 1993)). We have fixed the parameter $L_\infty = 218$ mm (Macer 1966) when fitting data (Fig. 3), as it is of minor importance for larval growth (because $L_m << L_\infty$), but L_∞ adds a little convexity to the growth curve. We notice

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Fig. 4. Comparison of observed and predicted larval length at catch using growth models 1–3 in combination with individual backtracking: (*a*) growth model 1; (*b*) growth model 2; and (*c*) growth model 3. The line in each figure corresponds to perfect agreement with observed–predicted larval length.

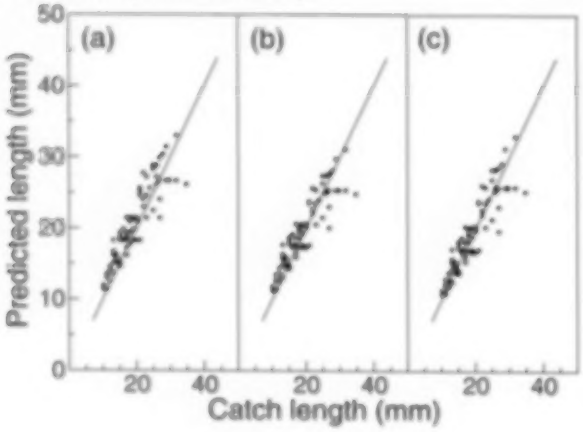
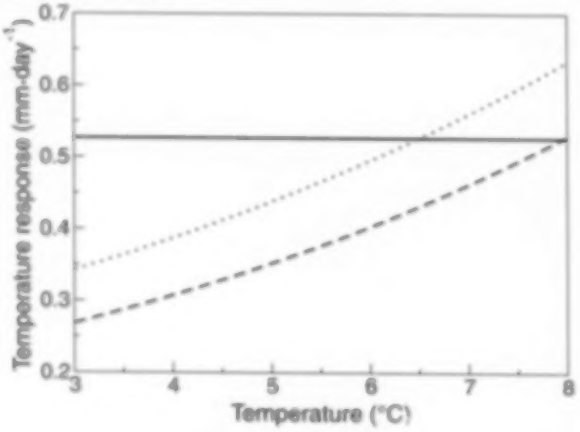


Fig. 5. Temperature response function $\lambda(T)$ for growth models 1–3: growth model 1, solid line; growth model 2, dotted line; growth model 3, broken line.



that the allometric term with $\gamma \neq 0$ becomes more important (sensitive) if growth in the postsettlement larval phase is considered.

The null model 1 (constant growth speed) already explains a very high degree of the data variance, leaving little room for improvement. Adding temperature and allometric effects may explain 20% of the residual variance only. Here, for premetamorphosed larvae, the temperature effects are relatively most important. This finding of a weak temperature influence is in accordance with Jensen (2001), who approached the problem indirectly via the correlation between otolith and larval growth. Here we confirm that indirect conclusion, considering the entire larval life history.

The variance structure in the residuals between the predicted and observed larval lengths (Fig. 4) is apparently the same in all models, supporting the statement that inherent individual variation and local environmental conditions beyond temperature influence is needed to account for the remaining residual variance. In the rest of this paper, we take growth model 3 as a reference model, which is used in all presented simulations, unless otherwise stated.

The temperature response functions $\lambda(T)$, inferred from the correlation between temperature track records and larval growth show that growth increases weakly with temperature (Fig. 5). The temperature derivative of the growth rate is of the order of 0.1 mm·day^{-1}·K^{-1}, in excellent overall agreement with Baistrocchi and Gallego (Baistrocchi 2003; Gallego et al. 2004), who estimated 0.13 mm·day^{-1}·K^{-1} from another data set in a linear growth–temperature regression. Similarly, the net larval growth is within 15% in the temperature regime relevant to presettled sandeel larvae. In a tank experiment for *Ammodytes americanus*, Smigielski et al. (1984) also found a positive temperature derivative of the order of 0.03 mm·day^{-1}·K^{-1}. Correcting for the fact that the absolute growth rate of this reared species sample grew at approximately one-quarter the overall speed of the displayed *Ammodytes marinus* sample, our temperature derivative is in accordance with their estimate. We note that part of the temperature response is likely indirect, via increasing secondary production with increasing temperature. Finally, we want to emphasize that generally determining the optimal separation of temperature response $\lambda(T)$ and the length scaling γ is difficult because seasonal temperature variation and larval length are strongly correlated. Currently, the growth model does not address explicitly density effects (food competition, cannibalism), although they are believed to affect recruitment on a population scale (Arnott and Ruxton 2002).

Larval premetamorphosis behavioural models

Horizontally, the larvae are described as passive drifters, with no explicit active vertical or horizontal migratory behaviour. Vertically, the dominant dispersal mechanism in the North Sea is turbulent diffusion, due to subgrid processes, coupled with current layer shear. The larvae move vertically in the water column according to a random walk (RW) process, with local step lengths reproducing local Eulerian field dispersal rates, which are proportional to the square root of the local diffusivity $K(x, y, z, t)$. This is modelled according to the following scheme (Visser 1997):

$$(7) \qquad dR = \frac{\partial K(z)}{\partial z} h + u\sqrt{6h \cdot K\left(z + \frac{h}{2}\frac{\partial K(z)}{\partial z}\right)}$$

where dR is a vertical RW step, h is the RW time step, and u is a uniform random number in $[-1, 1]$. This RW process mirrors the Eulerian process for small time steps h:

$$(8) \qquad \frac{\partial c}{\partial t} = \frac{\partial}{\partial z}\left(K\frac{\partial c}{\partial z}\right)$$

where $c(z, t)$ is the concentration of tracers. Encounter theory (e.g., in Fiksen and MacKenzie 2002) demonstrates biological advantages of actively selecting turbulence levels; this may be modelled by another gradient-driven term in eq. 7, similar to the first term in this eq. 7.

The boundary condition $dK(x, y, z)/dz = 0$ is imposed vertically at the surface and bottom to avoid artificial aggregation of tracers at the surface or bottom of the water column.

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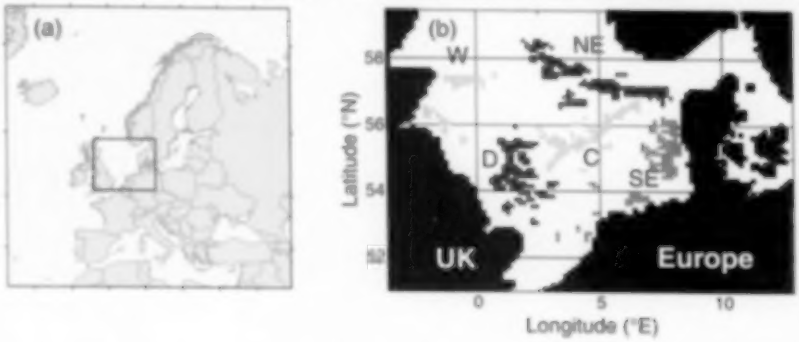
Fig. 6. (*a*) Study area shown as inlet. (*b*) Suitable sandeel banks in the central and southern North Sea divided into five major systems (s5). The bank systems are designated as shown in the figure: W, SE, D, C, and NE, which defines the enumeration order in the rest of the paper.



The simulations were performed with a time step $h = 30$ min using Euler forward integration. It was tested whether higher-order horizontal integration schemes (second-order Runge–Kutta) changed tracer trajectories, but the trajectories changed negligibly for $h = 30$ min. Longer time steps in conjunction with higher-order horizontal trajectory integration was not attempted, as this would imply large vertical jumps due to the stochastic modelling of turbulent dispersal. If tidal cycles were not averaged out of the hydrographic data, higher-order horizontal trajectory integration would be necessary to obtain accurate trajectories. In all presented spatial simulations, we used in total 600 000 RW tracers, which is sufficient for the current habitat resolution level and biological applications.

Sandeel habitats and settlement

During night and cold seasons with low zooplankton abundance, sandeel bury in the sandy seabed to avoid predation. The suitable sandeel habitats in the central and southern North Sea have recently been mapped (Fig. 6*b*) from Danish fishery data (Jensen and Rolev 2004).

Demersal eggs hatch at these sandbanks, and later larvae settle at these sandbanks at the end of their drift period, where they measure $L_m \sim 40$ mm. If metamorphosed juveniles do not migrate (which has not been observed), drift patterns must match habitat distributions to get successful recruitment; this is a central hypothesis examined in the present paper. Larvae not in the vicinity of a suitable sandbank at $L \sim L_m$ are assumed lost, because larvae loose transparency at metamorphosis, which strongly enhances the predation susceptibility.

Mapped sandeel habitats have been projected onto horizontal (5 nm × 5 nm) hydrodynamic cells, giving in total of 596 high-resolution habitat cells (the central and southern North Sea covers ~4200 cells, for comparison). To analyze patterns in population variability, these 596 high-resolution habitat cells are also clustered into five larger regional bank systems (Fig. 6*b*), and we denote this division s5 hereafter. Other more-detailed regional divisions are conceivable and well justifiable, but this generally places higher demands on our biological submodels, and assumptions and further large volumes of transport data tend to obscure the patterns present in the results. In particular, a much more fine-grained bank division may require a more detailed settlement model than the simple one used in this work. It is likely that habitat choice happens gradually around the length of metamorphosis $L = L_m \sim 40$ mm. This uncertainty is absorbed by choosing a coarse-grained bank system definition, because it is only boundary cases that are affected by this uncertainty: the transport indices are unaffected by uncertainties in settlement positions as long as they are in the correct bank system. The lateral extent of our bank systems is typically larger than 100 km, which analysis of similarity in sandeel size distributions among fishing banks has indicated as the maximum distance of subpopulation mixing (H. Jensen, unpublished data). No swimming longer than 10 km has been verified (K. Popp Madsen, Danish Institute for Fisheries Research (DTU-Aqua), Charlottenlund Slot, DK-2920 Charlottenlund, Denmark, unpublished data), which corresponds to the extent of a hydrodynamic cell, which is the spatial resolution limit in our study. Kunzlik et al. (1986) also inferred only minor horizontal migration from recapture experiments.

To assess the importance of a finite settling time window, we consider the two settling submodels. In the first model, we let larvae settle at length $L_m = 40$ mm (if they are at a suitable habitat); this settlement model is designated S40 in eq. 1. Secondly, we allow a finite settlement length interval, 37 mm $< L <$ 43 mm (at first encountered habitat, if any); this settlement model is designated flx in eq. 1. A larger time window (model flx corresponds to ~11 days) for settlement is unlikely, because larvae loose transparency at metamorphosis and becomes highly susceptible to predation. Of course, the flx submodel gives higher transport success, and this has to be weighted by the extra mortality incurred because of longer drift time.

Transport

We wish to quantify transport between banks and retention on banks. Unfortunately, the concept of transport is often used casually, so to clarify the meaning of transport, as well as the dependence of simulation parameters, we give a rather formal definition of transport indices (Fig. 7), in the sense that we use it in Appendix B.

Hydrodynamic transport of matter in a given current field is a linear process. This means, firstly, that transported matter is proportional to the released amount of matter and, secondly, that transport from one point to another is

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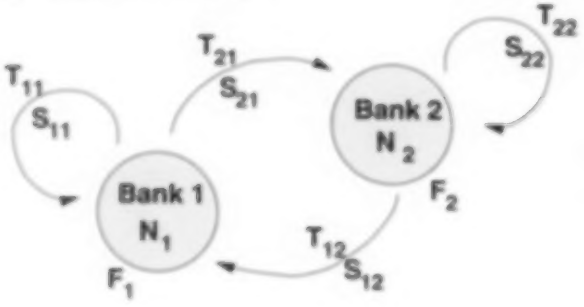
Fig. 7. Sketch of inter- and intra-bank transport dynamics: **T**, transport index of passive drifters; **S**, survival chance along transport paths; *F*, bank-specific egg productivity; *N*, adult sandeel population. Subscripts refer to banks.



independent of initial distribution of larvae. In other words, the net transport is a weighted superposition of point-to-point transport, where the weighting is the initial distribution of larvae. In Appendix B, we relate larval transport to the underlying hydrodynamic advection–diffusion fields and define larval transport indices $\mathbf{T}_{ij}^{hV}$ from bank j to bank i that only depends on hatch distribution $h(t)$ and the biological settlement model V used (for notational simplicity, we drop superscripts h and V when this clear from the context). In addition to transport success, the larvae also needs to survive the transport, and we describe this conditional probability by $\mathbf{S}$ ($0 < \mathbf{S} < 1$) as indicated (Fig. 7). Notice that bank retention is the diagonal terms $\mathbf{T}_{ii}$.

To analyze the transport structure, it is also convenient to consider three auxiliary contractions derived from the basic transport matrix $\mathbf{T}_{ij}$ characterizing regional transport. The first is the outflow ω_j from bank j successfully reaching other banks:

$$(9) \qquad \omega_j = \sum_{j \neq i} \mathbf{T}_{ij}$$

Adding bank retention to this gives the transport survival (or, in other words, the maximum potential for successful settling):

$$(10) \qquad \rho_j = \omega_j + \mathbf{T}_{jj}$$

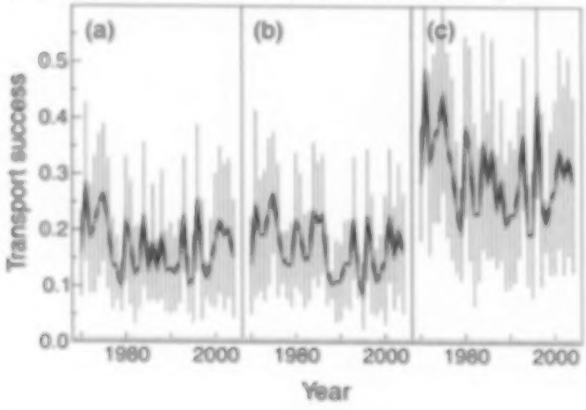
Finally, the inflow index from other banks to bank i is

$$(11) \qquad \nu_i = \sum_{j \neq i} \mathbf{T}_{ij}$$

We want to emphasize that our transport indices $\mathbf{T}$, ω_i, and ρ are independent of spawning intensity (and any uncertainty in spawning intensity assessment) because they describe the fraction of larvae that are successfully transported, due to linearity of transport, as discussed above.

Computationally, a transport matrix for a given year is obtained by releasing 600 000 tracers distributed homogeneously over all 596 hydrographic cells covering habitats and forward-tracking each of them by the appropriate biological submodel(s), as described above, until the juvenile stage; then the final positions are reprojected onto the 596 hydrographic cells covering habitats, and the raw 596 × 596 transport matrix is aggregated into the 5 × 5 regional transport matrix, corresponding to the area division *s*5 (Fig. 6*b*). With a 5 × 5 regional transport matrix as target, a sampling of the transport integral (eq. B4) with in total 600 000 tracers is sufficient for precision needed for biological applications.

Fig. 8. Interannual variability in transport survival ρ for all central and southern North Sea banks for alternative biological models, corresponding to Table 2: (*a*), model h_S40; (*b*), model e_S40; (*c*), model h_flx. Average transport survival is indicated by the solid line. Spatial standard deviation (i.e., bank variability) for a given year, referred to *s*5 bank division (Fig. 6*b*), is indicated by bars.



Conceptual spatial recruitment model

The transport indices defined above are cornerstones in computing annual recruitment from process-based modelling. Following the notation above (Fig. 7), the spatial explicit recruitment R_i^y in year y to bank i given by

$$(12) \qquad R_i^y = \sum_j (T_{ij}^y S_{ij}^y F_j^y) N_j^y = \sum_j \alpha_{ij}^y T_{ij}^y N_j^y$$

This equation arises from simply adding successfully transported larvae. T_{ij}^y is the transport index defined above, F_j^y is the bank-specific fecundity (for year y at bank j), and S_{ij}^y is the conditional survival factor for an interbank transport (bank $j \rightarrow$ bank i), given successful transport from bank $j \rightarrow$ bank i. So, by construction, $0 < S_{ij}^y < 1$. S_{ij}^y quantifies additional larvae loss due to starvation and predation. In eq. 12, we also have introduced α_{ij}^y, which we denote the productivity index for later use, with a clear relation to underlying biological processes, which enables further research. In practice, when modelling most importantly fecundity F_j^y, one must keep track of, e.g., age composition at each bank j. This is most conveniently done by keeping N_j^y from previous years y, when solving iteratively eq. 12, and we suppress this from eq. 12 for notational simplicity.

Results

We have calculated a 35-year time series of interbank transport for the central and southern North Sea as described above. The transport survival ρ for model h_S40 (reference model) shows significant interannual and spatial variability (Fig. 8*a*). The spatial variability (bars in Fig. 8) is depend-

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Table 3. Average (<T>) and standard deviation (RMS(T)) of transport survival for the years 1970–2004 for alternative larval models.

	<T>					RMS(T)				
	Scottish	SE	Dogger	Central	NE	Scottish	SE	Dogger	Central	NE
Model h_S40										
Scottish	0.055	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.001
SE	0.000	0.229	0.007	0.004	0.001	0.000	0.110	0.008	0.007	0.004
Dogger	0.013	0.000	0.264	0.019	0.000	0.017	0.000	0.088	0.034	0.000
Central	0.000	0.002	0.018	0.139	0.016	0.001	0.003	0.017	0.070	0.013
NE	0.029	0.007	0.000	0.012	0.041	0.035	0.010	0.000	0.015	0.023
Model e_S40										
Scottish	0.061	0.000	0.000	0.000	0.001	0.013	0.000	0.000	0.000	0.005
SE	0.000	0.225	0.008	0.004	0.001	0.000	0.107	0.011	0.007	0.005
Dogger	0.013	0.000	0.255	0.013	0.000	0.016	0.001	0.086	0.020	0.000
Central	0.001	0.002	0.019	0.135	0.011	0.003	0.004	0.013	0.076	0.010
NE	0.022	0.006	0.000	0.015	0.059	0.036	0.009	0.001	0.014	0.039
Model h_flx										
Scottish	0.112	0.000	0.000	0.000	0.001	0.029	0.000	0.002	0.000	0.003
SE	0.000	0.348	0.018	0.010	0.003	0.000	0.164	0.019	0.018	0.010
Dogger	0.022	0.000	0.420	0.034	0.000	0.027	0.001	0.117	0.056	0.000
Central	0.001	0.005	0.040	0.259	0.035	0.002	0.007	0.036	0.118	0.028
NE	0.057	0.023	0.000	0.028	0.086	0.066	0.034	0.000	0.032	0.044

Note: Rows and columns are indexed according to the regional division in Fig. 6*b*. Columns indicate starting bank system, whereas rows indicate transport destination.

ent, of course, on bank system partitioning, and the variability bars are based on the five major bank systems ($s5$) defined above (Fig. 6*b*). However, the average transport survival (Fig. 8, solid lines) is less sensitive to bank definition. The average transport success ($\langle\rho\rangle$) for this model is 17%, and the interannual standard deviation (RMS(ρ)) is 9.5%. The large spatial heterogeneity in the transport success stresses that spatial explicitness is necessary to approach robust recruitment models for North Sea sandeel larvae when comparing with eq. 12. For instance, in 1997, larvae starting on some bank systems only had 5% transport success, whereas other larvae of other subpopulations in 1997 had ~25% transport success. To compare $\langle\rho\rangle$ directly with VPA recruitment estimates would be premature, because the productivity index α_{ij} and population distribution time series N_j^* in eq. 12 also must be known to assess the recruitment. However, having the recent years of recruitment failure in mind, note that the time series (Fig. 8) does not reveal any recent climate change impacts on sandeel transport indices.

Transport structure

Statistical analysis of transport matrices and parameter sensitivity

We see that the overall trends and magnitudes in average transport indices are relatively robust toward reasonable changes in submodels that we have considered (Table 3) where the transport matrices $\mathbf{T}_{ij}$ corresponds to the $s5$ bank system (Fig. 6*b*). The transport matrices are quasi-diagonal (i.e., regional retention terms dominates). The region 3 (Dogger area) average transport retention dominates in all cases, of the order of 0.3.

The corresponding 35-year time series of transport survival ρ for the alternative models also show significant interannual and spatial variability (Figs. 8*b*, 8*c*). Comparing the influence of extended hatch period with a single, fixed hatch day on transport success (Table 3), we see that no systematic influences are evident, only small scattered differences, e.g., the transport minimum in h_S40 is higher compared with e_S40, whereas e_S40 has an extended period around 1990 with low transport success.

The model with flexible settling schedule (flx) has a generally higher level of transport survival (30%), but this is just a multiplicative scaling, which does not change qualitative predictions: the relative transport success between years corresponds to that in other models. The higher general level of transport success will, at least partly, be compensated for by increased mortality resulting from increased drift time. We will return to this issue in the discussion. Comparing simulations with growth models 1, 2, and 3 (Table 2), we see only marginal differences in all results, consistent with the comparable residual variance levels in growth models 1, 2, and 3 (Table 2). Therefore we report only results corresponding to growth model 3 in this paper.

To summarize, although some minor differences are present in the variance structure, it is seen that the interannual variability is also agreed on between model options considered: all models agree on which years are good and bad transport years, again demonstrating the point that the transport patterns are robust towards model choices.

It is clearly seen that larval influx and outflux constitute a minor part of the net survival (Fig. 9); this is a general pattern that holds across models and years. Some years of high transport success, such as 1975 and 1980, also display high inflow (ω) and outflow (ν), but later years, such as 2001, display negative covariation with retention. That retention dominates the survival is very dependent on how bank sys-

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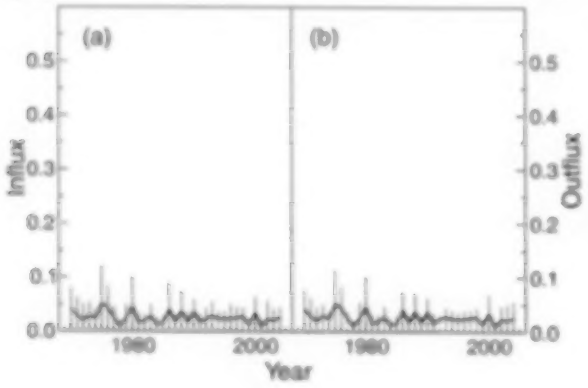
Fig. 9. (*a*) Influx index ν for $s5$ banks; (*b*) outflux index ω shown as time series averaged over banks for reference model h_S40. Bank variability referring to $s5$ bank division in Fig. 6*b* is indicated by bars.



tems are defined: defining smaller bank systems generally shifts the reason for transport success from retention to successful influx and outflux. However, the net survival is relatively insensitive to how banks are aggregated into bank systems.

Spatial and temporal covariation in transport matrices

We have also tested the transport indices for spatiotemporal covariance patterns by evaluating the lagged pair covariance estimator:

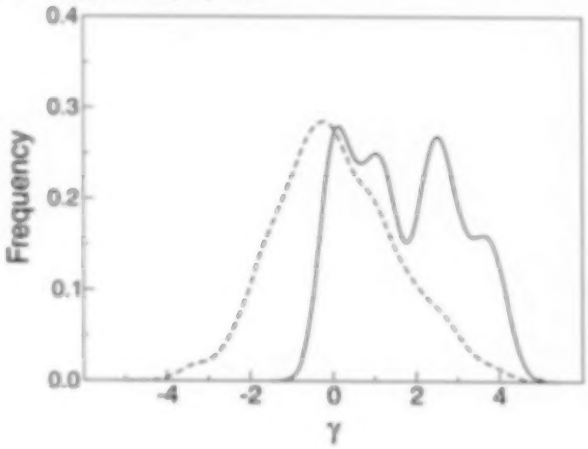
$$(13)\qquad C^{m}_{ij,kl} = \frac{1}{1 + Y_1 - Y_0 - m}\sum_{y}(T^{y}_{ij} - \langle T^{y}_{ij}\rangle_y) \times (T^{y+m}_{kl} - \langle T^{y}_{kl}\rangle_y)$$

for our generated time series of transport matrices covering years $Y_0 = 1970 \le y \le Y_1 = 2004$. Here m is time lag in years and brackets $\langle \ldots \rangle_y$ indicate time average of the quantity in the bracket. It is seen that $C^{m}_{ij,kl} = C^{m}_{kl,ij}$, so we consider only time lags $m \ge 0$, but all combinations of $[ij;kl]$. However, even with our coarse regional bank division $s5$ (Fig. 6*b*) containing five regions, an immense number of covariance possibilities exist, especially if large time lags m are also considered. Two classes of covariances are especially interesting: spatial covariance within the same year ($C^{m}_{ij,kl}$) and transport autocorrelation ($C^{m}_{ij,ij}$). To test the statistical significance of the transport covariances, we divided by the standard deviation $\sigma(C^{m}_{ij,kl})$ of the estimator $C^{m}_{ij,kl}$ evaluated analytically for the null hypotheses that T^{y}_{ij} are independent stochastical variables (in this case, the estimator $C^{m}_{ij,kl}$ can be expressed in terms of second and fourth moments of the transport matrices, which can be estimated directly from the transport matrix time series). We term this the excess covariance:

$$(14)\qquad \gamma^{m}_{ij,kl} = \frac{C^{m}_{ij,kl} - \langle C^{m}_{ij,kl}\rangle}{\sigma(C^{m}_{ij,kl})}$$

Additionally, because of the large number of covariance $[ij;kl]$ index combinations, the level of statistical significance must be adjusted down by the Bonferroni correction to avoid statistical type II errors (false positive statistical test compared with the null hypothesis). People often use less conservative measures than the Bonferroni correction to screen statistical type II errors, but we do not want to enter this dubious path of arguing statistical acceptance limits without indisputable reasons. All covariances ($m = 0, 1$) and all $[ij;kl]$ were tested for statistical significance.

Fig. 10. Distribution of excess covariance for reference model h_S40: covariance of retention terms with time lag 0, solid line; distribution of all excess covariances with time lag 0 and 1 year, all pooled together, broken line. Distributions were broadened with $\gamma = 0.4$ to suppress sampling noise.



The excess distribution for all covariances (Fig. 10, broken line) is close to a normal distribution, indicating that the bulk the covariances $\{C^{m}_{ij,kl}\}$ are indeed explained as random fluctuations. A single class of covariances significantly exceeded 1% Bonferoni corrected significance level: the $m = 0$ positive correlation between bank retention (Fig. 10, solid line). This was observed across all tested models. The most significant covariation was between SE–Dogger and Dogger–central retention. A single negative correlation involving interbank transport emerged: SE–Dogger and Dogger retention, which were marginally significant. These findings make sense from the point of view that retention and interbank transport are complementary, to some extent, if the net transport survival does not vary too much. So if interbank transport is low, retention should be high and correlated over many regions. Interbank transport is less correlated, because many coupling correlations exist. The complementary interpretation is also emphasized by the negative SE–Dogger and Dogger retention covariation.

In summary, in the time series underlying this work, the spatiotemporal covariances of matrices T^{y}_{ij} are generally weak or nonexistent. Only retention elements show some positive covariation. Therefore, at a first course level of modelling, we conclude that it is a reasonable approximation to consider T^{y}_{ij} as independent stochastic variables from a nonnegative distribution with average and standard deviation as given (Table 3). The next step in stochastic modelling is to include the retention covariation, which can be implemented relatively easily.

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Discussion

Our transport indices T_{ij}^{γ} may be applied directly into sandeel recruitment models, as demonstrated above. First, however, the spatial and interannual heterogeneity of F_j^{γ} and S_{ij}^{γ} must be estimated to produce realistic recruitment predictions. A realistic parameterization of F and S is beyond the scope of the present study, but we would like to add a few remarks aiding further investigations in this direction. A very interesting question is whether transport indices alone are able to explain recruitment variability (i.e., we may assume $\alpha = S \cdot F$ is relatively constant in eq. 12). This question may be addressed at a very crude level, if we consider the recruitment per standing stock number (SSN): $r = R/N = \alpha T$. The final International Council for the Exploration of the Seas (ICES) ACFM sandeel assessment (1983–2004) (ICES 2005) shows that recruitment per SSN displays the relative variability $\sigma(r)/\langle r \rangle \sim 0.5$. Using standard rules of probability calculus and our results in this paper, we obtain the rough order-of-magnitude estimate:

$$(15) \qquad \frac{\sigma(\alpha)}{\langle \alpha \rangle} \approx \frac{\sigma(T)}{\langle T \rangle} \approx 0.3$$

where an adult total average mortality $z \sim 1.5\text{-year}^{-1}$ from MSVPA (ICES 2007) has been applied. In other words, productivity α and transport T are about equally important in determining recruitment variability, and to make quantitative recruitment predictions, both α and T must be addressed. This simple observation also explains why previous North Sea transport studies have not been able to consistently explain recruitment variability. We also want to stress that an implication of eq. 12, the spatial heterogeneity in transport success, and the correlation analysis of T_{ij}^{γ} time series is that deterministic recruitment models must be spatially explicit to approach goals of being quantitatively reliable. From eq. 12, we may sum up the total stock–recruitment:

$$(16) \qquad \begin{aligned} R^{\gamma} &= \sum_{ij} \alpha_{ij}^{\gamma} T_{ij}^{\gamma} N_j^{\gamma} \\ &= \left(\sum_{ij} \alpha_{ij}^{\gamma} T_{ij}^{\gamma} \frac{N_j^{\gamma}}{N_{\mathrm{SSN}}^{\gamma}} \right) N_{\mathrm{SSN}}^{\gamma} \\ &= Q^{\gamma} N_{\mathrm{SSN}}^{\gamma} \end{aligned}$$

where Q^{γ} is the (density-dependent) recruits per spawner. A nonspatial recruitment model requires that fluctuations in α_{ij}^{γ}, T_{ij}^{γ}, and N_j^{γ} are proportional, and there is no reasonable basis for expecting this, following our analysis in the previous section. Following traditional stock–recruitment models and imposing $Q^{\gamma} = Q(N_{\mathrm{SSN}}^{\gamma})$ leads to a stochastic function $Q(N_{\mathrm{SSN}}^{\gamma})$, the variance of which ultimately limits the predictive power of the recruitment model. These considerations hold for sandeel and may be extended to other species for the same reasons.

In our formulation, any mortality schedule can be applied a posteriori to the transport calculation, which is a great advantage when comparing scenarios and assessing parameter sensitivity. A reasonable null model is to use a spatially uniform S^{γ}, which is justified by the fact that the passive drift time of survivors is reasonably uniform, as implicated from the variance analysis of growth data underlying the present study. A next step would be to overlap drift trajectories with temporal and spatially resolved effective predation fields. In this context, it would be interesting to assess the influence of herring, which is known to prey on sandeel larvae and early juveniles (Pommerantz 1981; Hopkins 1989; Last 1989). However, adult sandeel has also been identified as a main inhibitor on sandeel recruitment (Daan et al. 1990; Kimura et al. 1992; Arnott and Ruxton 2002). Whether this interaction is competition for food or habitat between larvae and adults or cannibalism is a question that is as yet unanswered.

We found that the temperature effect on sandeel growth was relatively moderate and of a magnitude consistent with other relevant studies of sandeel. It is important to realize that our conclusions pertain to survivors. We do not currently know much about the ones that starved along transport and the effect of starvation on survival, but this is necessary to determine the starvation component of S. Generic bioenergetic growth models (Letcher et al. 1996) stipulate that larvae die if they loose more than 25% somatic weight in starvation periods. Such conditions generate sharp windows of survival, when stochastic effects are included (e.g., small-scale patchiness), and therefore we have reason to believe that the larval sensitivities to ambient biological parameters are strong and complex, as it is difficult to determine the exact edges of the survival window from survivorship analysis. Because larvae posses elementary regulatory mechanisms (e.g., to keep on searching for food until satiated) and, at the same time, have hard biological limits (e.g., death when weight loss exceeds 25%), it is an interesting question how much survival is actually controlled by smooth spatial variations in ambient biological conditions, compared with inevitable small-scale fluctuations in ambient biological conditions.

Another issue for further investigation is the effect of active vertical and horizontal larval behaviour, including subdaily motion, such as diurnal migration (Jensen et al. 2003), where larvae appear to actively seek vertical layers with high zooplankton abundance in light periods. It is also an open question whether large-scale horizontal food gradients influence larval transport indirectly by biasing local food-seeking activity. Also, passive hydrodynamic mechanisms for frontal retention (Franks 1992) must be examined in closer detail for the North Sea. The significance of vertical migrations on horizontal transport has been exemplified for zooplankton in other areas (Queiroga and Blanton 2004). The cues for vertical migration could be tidal or ambient light signals. The importance of such hypotheses will be examined in future work, but unfortunately, it is not possible to address subdaily motion with the present hydrodynamic database, because physical fields are averaged to daily average fields. This would require a tidal current correction to the daily average fields in conjunction with a pattern of active subdaily motion.

An interesting finding in our study was that a flexible settlement schedule (settlement over a week) compared with a more tight settlement schedule gave the strongest increase in overall transport success (from 17% to 30%). However, this has to be balanced against a possible increased risk of predation (due to loss of transparency at metamorphosis). The

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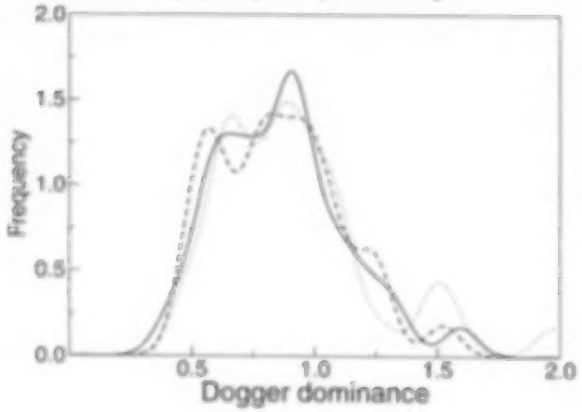
Fig. 11. Probability distributions of dogger area growth dominance over competing areas: model h_S40, solid line; model e_S40, dotted line; model h_flx, broken line. Dogger growth dominance indicated by the ratio between the largest non-Dogger eigenvalue (i.e., the corresponding eigenvector has its main weight on another region) and the eigenvalue belonging to Dogger. When the ratio is around 1, there is a propensity for "spatial blinking".



exact risk increase is unknown, but it is reasonable to assume that it is on the scale of the mortality z experienced at that life stage. A rough estimate is $z \sim 0.07\text{-day}^{-1}$ (Wright and Bailey 1996). Adding this up to a week decreases the survival chance by 40%. So we have good reason to believe that the increased survival chance by flexible settling schedule is more or less cancelled by a corresponding decrease in conditional survival factor S. This observation strengthens the validity of our calculated transport indices. We have not considered the effect on transport success of spawning season width in the present study; however, in comparison with the minute effect of a prolonged hatch period (initialization model e versus model h), we believe that this will have a minor impact on the results. The same argument also holds for small changes in the offset of the hatch period.

In this paper, we have considered a relatively coarse regional division of the North Sea sandeel habitats. However, with the raw, high-resolution transport matrices $\mathbf{T}_R$ (i.e., the 596 × 596 transport matrix) computed in this study, it is always possible to calculate the transport for any bank system definition alternative to s5 by a simple transformation:

$$\mathbf{T} = \mathbf{U}^{\mathrm{T}}\mathbf{T}_R\mathbf{U}\boldsymbol{\pi} \tag{17}$$

which is derived from the transport integral definition (eq. B4). The matrix $\mathbf{U}_{ik} = 1$ if cell i is a member of bank k, and 0 otherwise. $\boldsymbol{\pi}$ is a diagonal matrix of priors, usually, e.g., the inverse of the number of cells in bank k. However, a significantly finer regional division requires an increased number of tracers to resolve transport between weakly connected regions properly, because of stochastic sampling noise.

The considerations in the previous section focused primarily on statistical aspects of interbank transport. However, the quantitative and even qualitative demographic consequences are not obvious from this. For instance, stochasticity may trigger spatial or temporal resonances (McKane and Newman 2005) in the population structure. A central element in this respect are the eigensolutions $[\mathbf{w}_k, t_k]$ of the transport matrices:

$$\mathbf{T}\mathbf{w}^k = t^k\mathbf{w}^k \tag{18}$$

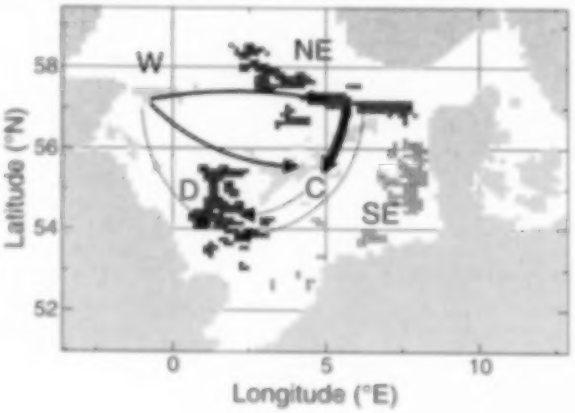
The solutions to this equation describe population distribution modes being amplified by the transport structure. In the following, we index eigensolutions according to descending modulus $|t^k|$. Assuming that the productivity index σ is spatially relatively weakly varying, the transport will amplify a spatial population distribution that is parallel to the leading eigenvector w^0, if the leading eigenvalue is well-isolated, i.e., $|t^0/t^1| >> 1$. For all submodels considered in this paper, we find that the leading eigenvector, on average, has its main weight for the Dogger region (region 3). This is simply driven by the fact that this region has the largest retention on average. It is interesting to investigate the temporal stability of this, i.e., whether the stability flips occasionally. This is indicated by distribution functions of the ratio between the largest non-Dogger eigenvalue (i.e., the corresponding eigenvector has its main weight on another region) and the eigenvalue belonging to Dogger (Fig. 11). The distribution functions are generated from the eigensolutions of each transport matrix in our calculated time series. When the dominance ratio (Fig. 11) exceeds 1, the stability flips to another region. We see that there are some differences on this aspect between our considered submodels, but all considered models agree that in about two-thirds of the years, Dogger is dominating, whereas in about

Table 4. Expected travel times (t, in years) between regions for model h_S40.

	Start				
End	1	2	3	4	5
1	—	>100	>100	>100	>100
2	44.9	—	41.7	42.3	43.0
3	18.8	49.5	—	18.7	23.9
4	12.3	31.5	20.5	—	5.4
5	16.7	35.9	45.8	33.9	—

Note: Columns are starting regions; rows indicate destination regions (see Fig. 6b).

Fig. 12. Most important interbank exchanges in the North Sea of sandeel larvae between s5 regions (Fig. 6b).



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one-third of the years transport favours other bank populations. Notice that these considerations are independent of the absolute values of α, to the extent that they are spatially homogeneous.

Finally, we want to discuss the bank connectivity structure implied by the transport matrices {**T**} in a little more detail. Essential properties are whether there exist isolated subpopulations, larval sources, or sinks. To answer these questions qualitatively, it suffices to focus on larval transport. We are not interested in the advective larval loss, i.e., larvae advected to areas without suitable habitats in which to settle. Therefore, we renormalize column-wise the transport matrix to turn it into a proper Markov matrix **P**. This renormalization conserves the bank connectivity and relative efflux rates, which is essential for investigating dispersal time scales. Alternatively, only retention terms could be renormalized; both procedures give the same qualitative picture. The question of whether isolated subpopulations exist or not is then turned into a stringent question about whether **P** is regular or not. A sufficient condition (e.g., Bartlett 1978) for regularity is that all elements of the matrix $\mathbf{Q}^M$,

$$\mathbf{Q}^M = \sum_{m=1}^{M} \mathbf{P}^m \tag{19}$$

are nonzero for some $M > 0$. Loosely speaking, this condition tests whether it is possible to travel between all banks in, at most, M steps. (In other words, this condition also detects banks that are indirectly transport-connected, which may not be evident from the transport **T** alone. To detect strict isolation of certain regions, we need only take $M = 5$ in eq. 19 above, as the longest nonoverlapping, indirect pathways connecting regions have then been exhausted.) We find that $\mathbf{Q}^5$ is strictly nonzero for all submodel combinations considered in this paper; however, $\mathbf{Q}^M$ also indicates that regions are coupled to each other at different strengths. To elucidate this aspect, we introduce Y_{ij}, which is the expected time to travel from bank j to bank i, measured in years, when individuals are exchanged according to the Markov matrix **P**. In Appendix C, we show that **Y** can be calculated by the matrix equation:

$$\mathbf{Y} = (\mathbf{1} - \mathbf{I}) \cdot \{(\mathbf{1} + \mathbf{Y})\mathbf{P}\} \tag{20}$$

where **1** is a matrix of ones, **I** is the matrix of unity, and the centered dot ($\cdot$) denotes an element-by-element matrix product.

The expected travel time (Table 4) corresponding to transport indices of the reference model h_S40 (Table 3) pertains to spreading time of, e.g., a passive genetic mutation (but time scales should be corrected for detection level). All models considered in this paper display the same approximate ordering and magnitude of the travel times (Fig. 12), even though some quantitative differences exist. Generally, s5 regions are weakly coupled, the expected travel times being several years. Travel times from northern banks to central banks are smallest. The Scottish side banks act as sources for the rest of the North Sea (most dominantly the central and northern banks), the return times being extremely large. There is also a relatively large coupling between Dogger and central banks. The remaining expected travel times are typically of order of 30–40 years. The huge return times to Scottish banks should not be taken very literally; these numbers are based on the average transport matrices — if interannual variations were included, these numbers would drop significantly, but still be large. Transport between weakly connected regions will be dominated by occasional bursts.

We also note that the exchange pattern is highly asymmetric (being directed). These predictions constitute an important contact point for population genetic investigations.

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Appendix A. Parameterization of hatch probability function

At a given temperature T, the hatch process is characterized by three time scales, measured from the spawning point: $b(T)$ is the hatch beginning, $m(T)$ is the hatch median, and $e(T)$ is the hatch end point. Imposing normalization and median condition on the probability distribution eq. 3 gives

$$g(T) = \frac{b(T)e(T) - m(T)^2}{b(T) + e(T) - 2m(T)} \quad \text{(A1)}$$

$$n(T) = \ln\frac{e(T) - \gamma(T)}{b(T) - \gamma(T)} \quad \text{(A2)}$$

If the hatch distribution is skewed toward the hatch beginning, then $g(T) < b(T)$. In the special case in which the hatch probability becomes uniform, $m = (b + e)/2$, $\gamma(T)$ becomes large.

Appendix B. Definition and uniqueness of larval transport indices

The function describing the point-to-point transport is the so-called Greens function (or transport kernel), $G(x, t, x', t')$, which describes the probability of being transported from (x', t') to (x, t). If we only included hydrodynamic advection, $G(x, t, x', t')$ would be peaked at the point (x, t) given by following the current line through x' at time t' until time t. If turbulent diffusion is included, $G(x, t, x', t')$ will be an expanding patch around the advection locus (x, t) (Fig. B1). The function $G(x, t, x', t')$ is completely and uniquely speci-

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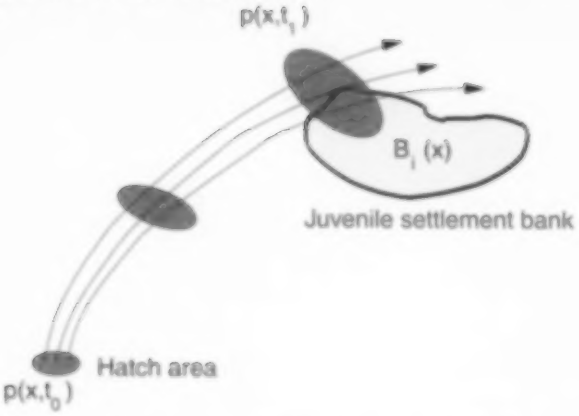
Fig. B1. Sketch of larval advection.



fied from the hydrodynamic advection–diffusion in the region of interest.

If we denote an initial larval patch distribution at time t_0, $p(x', t_0)$, later at time t_1, it has been transported into a patch distribution according to

$$p(x, t_1) = \int G(x, t_1, x', t_0) p(x', t_0) \mathrm{d}x' \tag{B1}$$

Rather than mapping the full transport Greens function $G(x, t, x', t')$ (which is an eight-dimensional field), we focus on bank-to-bank transport described by $G(x, t, x', t')$. A bank i is described by a shape function $B_i(x)$, which is 1 if x is on the bank and zero elsewhere. The bank also has an area A_i (which can be obtained by integrating $B_i(x)$). Thus, if banks i and j are described by the shapes $B_i(x)$ and $B_j(x)$, respectively, then we describe the transport matrix $\mathbf{T}_{ij}$ by

$$\mathbf{T}_{ij}(t_1, t_0) = \frac{1}{A_j} \int \int G(x, t_1, x', t_0) B_j(x') B_i(x) \mathrm{d}x \mathrm{d}x' \tag{B2}$$

The factor $(1/A_j)$ is a prior that assigns equal weights to all positions within the starting bank system j (which is a reasonable null hypothesis in the absence of more detailed knowledge and when the spatial extent of the bank system is small or moderate). For the regional splitting s5 in the present work, $i, j = 1, \ldots, 5$.

We let the settling time t_1 be determined by the biological clock, namely where the larvae reach the length of metamorphosis at $L \sim 40$ mm (Macer 1966; Reay 1970; Wright 1993). The probability that a larvae described by a biological model V attempts to settle at a given time t_1 depends on the position x and starting position and time (x', t_0) as

$$P(t = t_1 | x, x', t_0) = V(t_1; x, x', t_0) \tag{B3}$$

The settlement probability V depends on the transport history and is rather peaked in time. For simple biological models (like the one in this paper), V can be evaluated by path integral techniques, if the transport is computed by a Eulerian approach. We effectively compute V by a Monte Carlo approach when we run our combined hydrodynamic IBM. Inserting V into eq. B2, we obtain the settling probability on bank i from bank j as

$$\begin{aligned} \mathbf{T}^V_{ij}(t_0) &= \int \int \mathrm{d}x \mathrm{d}x' B_i(x) B_j(x') \int \mathrm{d}t_1 G(x, t_1, x', t_0) V(t_1; x, x', t_0) \\ &= \int \int \mathrm{d}x \mathrm{d}x' B_i(x) B_j(x') \Omega^V(x, x', t_0) \end{aligned} \tag{B4}$$

Thereby we have defined a transport index $\mathbf{T}_{ij}(t_0)$ that depends only on initial release time and the biological model V used; we also have derived rigorously the bank connectivity kernel Ω in terms of well-defined hydrodynamic and biological fields, as well as the exact parameter dependence of Ω.

If the hatch time is extended and described by a probability distribution $h(t)$, the net transport (over the season) is computed as a weighted integral, again due to linearity of transport:

$$\mathbf{T}^{\text{tot}}_{ij} = \int \mathbf{T}^V_{ij}(t) h(t) \mathrm{d}t \tag{B5}$$

Appendix C. Expected travel time between bank systems

We want to calculate the average number of steps $\mathbf{Y}_{ij}$ required to travel from sites j to i. The probability to travel from sites j to i in one step (in our context, the step unit is a year) is designated $\mathbf{P}_{ij}$, and especially important, the retention probability $\mathbf{P}_{ii}$ can be nonzero. The expected travel time is most elegantly derived by a recursive argument:

$$\mathbf{Y}_{ij} = \begin{cases} \sum_k (1 + \mathbf{Y}_{ik}) \mathbf{P}_{kj} & i \neq j \\ 0 & i = j \end{cases} \tag{C1}$$

which expresses that the travel time is the weighted sum of direct and indirect travel paths times + 1. This recursive relationship can be written compactly as eq. 20, which is most conveniently solved iteratively (i.e., inserting a guess on right side, evaluating the equation, reinserting the result on right side, etc., until converged), but it can also be solved directly element by element, if desired. Notice that the travel time matrix $\mathbf{Y}$ is not symmetric in general.

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A management framework for preventing the secondary spread of aquatic invasive species

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Abstract: Biological invasions continue to accelerate, and there is a need for closer integration between invasive species research and on-the-ground management. In many regions, aquatic invasive species have established isolated populations, but have not yet spread to many sites that provide suitable habitat. In the Laurentian Great Lakes region, several Great Lakes invaders such as zebra mussel (*Dreissena polymorpha*), rainbow smelt (*Osmerus mordax*), and spiny water flea (*Bythotrephes longimanus*) are currently undergoing secondary spread to the smaller inland lakes and streams. This paper describes recent advances in forecasting the secondary spread of aquatic invasive species and presents a framework for assessing vulnerability of inland waters based on explicit assessment of three distinct aspects of biological invasions: colonization, site suitability, and adverse impact. In many cases, only a fraction of lakes on the landscape are vulnerable to specific invasive species, highlighting the potential application of this type of research for improving invasive species management. Effective application to on-the-ground resource management will require that research aimed at assessing site vulnerability be translated into management tools.

Résumé : Les invasions biologiques continuent à augmenter en nombre, c'est pourquoi il est nécessaire de mieux intégrer la recherche sur les espèces envahissantes et leur gestion sur le terrain. Dans plusieurs régions, les espèces aquatiques envahissantes ont établi des populations isolées, mais ne se sont pas encore propagées dans plusieurs sites qui constituent des habitats appropriés. C'est le cas de la région des Grands Lacs laurentiens où des espèces qui ont envahi les Grands Lacs, telles que les moules zébrées (*Dreissena polymorpha*), l'éperlan arc-en-ciel (*Osmerus mordax*) et la puce d'eau épineuse (*Bythotrephes longimanus*), sont actuellement en train d'étendre secondairement leur répartition aux petits lacs et cours d'eau de l'intérieur des terres. Notre travail fait la synthèse des développements récents dans la prédiction de la dispersion secondaire des espèces aquatiques envahissantes et présente un cadre pour évaluer la vulnérabilité des eaux intérieures d'après une détermination explicite de trois aspects différents des invasions biologiques, soit la colonisation, la convenance des sites et l'impact négatif. Souvent, seule une fraction des lacs dans un paysage sont vulnérables à une espèce envahissante donnée, ce qui démontre l'intérêt potentiel de ce genre de recherche pour l'amélioration de la gestion des espèces envahissantes. Une application efficace à la gestion des ressources sur le terrain requerra cependant que la recherche sur l'évaluation de la vulnérabilité des sites soit incorporée dans des outils de gestion.

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Introduction: the challenge of invasive species management

In this age of globalization, human activities are rapidly dissolving the natural barriers that have historically isolated animal and plant species, providing vast opportunities for species to become biological invaders (Elton 1958). Invasive species are a leading threat to native species and biodiversity (Wilcove et al. 1998; Sala et al. 2000), are an important driver of global ecological and evolutionary change (Myers and Knoll 2001; Olden et al. 2004; Lodge et al. 2006), and have caused significant economic damage (Pimentel et al. 2005). Freshwater ecosystems are especially vulnerable to biological invasions and species extinctions because of their high degree of isolation and endemism (Richter et al. 1997; Ricciardi and Rasmussen 1999; Dudgeon et al. 2006). In response, many governments are developing management strategies for prohibiting new introductions and reducing the impacts of aquatic invasive species. The task is daunting: landscapes to be managed are vast, there are numerous invasive species, and resources available for invasive species management activities are limited.

Because biological invasions are generally irreversible, invasive species prevention is the cornerstone of many man-

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Table 1. Comparison of two invasive species prevention strategies: preventing new species from being introduced in the first place and preventing or slowing the secondary spread of invasive species that have already established regionally.

	Preventing introduction	Preventing secondary spread
Goal	To keep out potentially invasive species	To prevent or slow the further spread of invasive species in a region
Geographic focus	Borders, point-of-entry, ballast water	Diffuse on landscape, invasion hubs, vulnerable sites
Approach	Legislative: prohibit trade and transport of potential invaders, border inspection	Education and outreach to the general public
Target audience	Shippers, industry, importers	Recreational boaters, vacationers, general public
Taxonomic focus	Many potential species, identity of species often unknown	Small number of species, identity of species known

Note: This comparison is based on the experience in the Laurentian Great Lakes, which currently support a large number of non-native species and also acts as a source habitat for the secondary spread of invasive species to smaller inland waters in the region.

agement strategies (Ruesink et al. 1995; Mack et al. 2000; Simberloff 2003). The term "prevention" generally refers to prohibiting the entry of potentially invasive species into a country or new range, as conventional wisdom dictates that once a new invasive species establishes, it is usually impossible to eradicate or prevent its subsequent spread (Table 1). A number of national regulations and international agreements govern the movement of potentially invasive or harmful species, and protocols have been developed for assessing potential harm or invasiveness of a species being considered for import (Hayes 2003; Orr 2003; Lodge et al. 2006). More recently, quantitative approaches have been used to identify species most likely to become invasive (Ricciardi and Rasmussen 1998; Kolar and Lodge 2002; Marchetti et al. 2004). Although preventing the introduction of new invasive species is a critically important task, this approach represents only one aspect of the broader concept of invasive species prevention. Many potentially harmful invasive species have established populations outside of their native range, yet may be far from achieving their potential geographic distribution. Preventing or slowing the secondary spread of known and established invasive species to uninvaded sites is an additional aspect of invasive species prevention (Table 1) and involves a different set of goals, strategies, and target audiences than efforts to halt the import of new species (Table 1).

To explore the issue of secondary spread of aquatic invasive species, we draw upon examples in the Laurentian Great Lakes region of North America. The number of non-native species that have invaded the Great Lakes now exceeds 180, and new invaders continue to establish, primarily as the result of ballast water discharge from transoceanic vessels (Holeck et al. 2004; Ricciardi 2006). A subset of these Great Lakes invaders is spreading into the small inland lakes and streams in the surrounding region, often as unintentional hitchhikers on recreational and fishing boats. In effect, the Great Lakes are acting as stepping stones for these invasive species to reach the many thousands of ecologically diverse, smaller inland waters of North America. Inland lakes are relatively discrete ecosystems that are physically separated by land, habitat that is inhospitable to aquatic species. As a result, overland dispersal of these invasive species has been slow, and many suitable ecosystems have remained uninvaded for long periods (Johnson et al. 2006). In addition, inland lakes vary widely with regards to suitability to invasive species, a function of their individuality in physical, chemical, and biological attributes. Though the invasion of a single, small, inland lake is a relatively minor event, the potential impact of Great Lakes invasive species on small inland water bodies may be vast for several reasons. First, the Laurentian Great Lakes region contains at least several hundred thousand small lakes, along with hundreds of thousands of kilometres of rivers and streams on both sides of the Canada–US border. These inland waters provide a suite of valuable ecological services and have high recreational and ecological value (Wilson and Carpenter 1999). Many are heavily used by humans and are preferred sites for recreation and residential development (Schnaiberg et al. 2002). Current and potential economic and ecological impacts of Great Lakes invasive species on these inland water bodies are poorly characterized, though the cumulative magnitude of these impacts may be tremendous.

The sheer number of inland water bodies in the Laurentian Great Lakes region, as well as many regions of the world, presents an enormous invasive species management challenge. In light of limited management resources, knowledge of which sites are vulnerable to specific invasive species is valuable because it can help direct management efforts to where they are likely to provide the greatest benefit with respect to minimizing undesired impacts of introduced species. Some of the earlier invasive species studies considered invasibility as a general attribute of a site, posing questions such as whether altered disturbance regimes and anthropogenic nutrient enrichment rendered sites more invasible (Orians 1986; Moyle and Marchetti 2006). More recent research recognizes the importance of multiple interacting factors in determining the outcome of invasions: propagule pressure, the match between site attributes and species requirements, and biotic interactions with native taxa (Kolar and Lodge 2002; Vander Zanden et al. 2004*a*).

This paper examines the interface of research and resource management aimed at preventing or slowing the secondary spread of freshwater invasive species. There has been a great deal of progress in recent years in understanding and modeling aquatic invasive species on landscapes, specifically with regards to propagule introduction, site suitability, and impact. Each of these three factors represents a critical component of the biological invasion process, and each can contribute to assessment of site vulnerability. Identifying the specific sites on a landscape that are vulnerable to invasive species has the potential to help guide invasive species prevention programs to the locations where manage-

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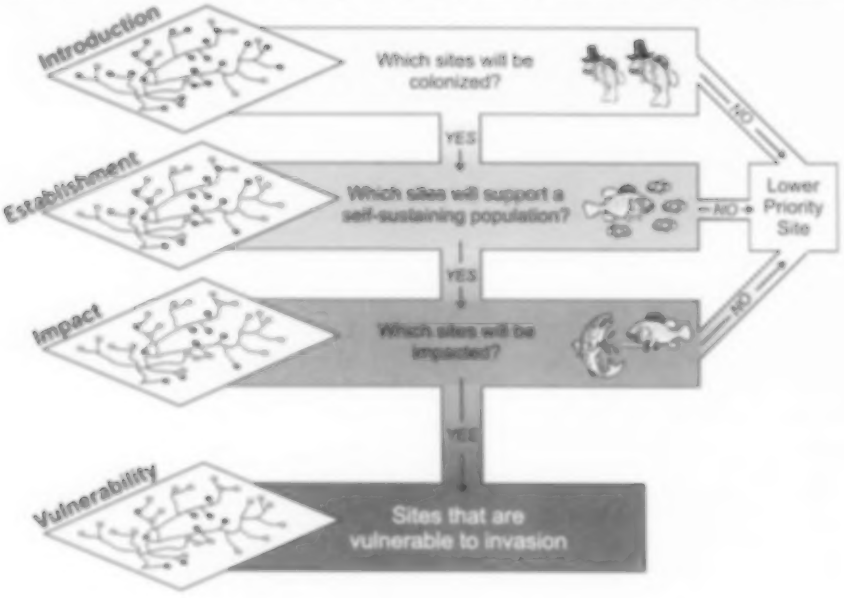
Fig. 1. A conceptual framework for assessing site vulnerability on a landscape comprised of multiple lakes. The approach separately assesses potential for introduction, establishment, and adverse impact of a specific invasive species for each of a suite of lakes. Assessment of vulnerability for individual lakes can help guide the targeting of invasive species prevention and management efforts.



ment and prevention are expected to produce the greatest benefit. We refer to the application of this research to on-the-ground management as "smart prevention" of invasive species.

Modeling site vulnerability to freshwater invasive species

Our approach to assessing site vulnerability explicitly considers each of three discrete stages or filters that need to be overcome for an invasion to occur and be of management concern (Fig. 1). The first stage is whether viable propagules or colonists will reach a site of interest (arrival). The second is whether colonists will establish a self-sustaining population by surviving environmental conditions and interactions with residents of the community and successfully reproduce (establishment). The third is whether the species will have adverse ecological or economic impacts (impact). This conceptual approach for assessing vulnerability to a hypothetical invasive species on a landscape comprised of multiple lakes is illustrated (Fig. 1). The framework provides a basis for classifying sites according to vulnerability, which reflects the risk of invasion and impact from a specific invasive species. Geographic information systems (GIS) provide a tool for mapping and visualizing ecosystem vulnerability on the landscape. In the following sections, we highlight advances in modeling each of these three stages using examples from the Laurentian Great Lakes region.

Introduction: can colonists get there?

Several authors have identified propagule pressure as a central predictor of invasions (Williamson 1996; Colautti et al. 2006*a*), though the predictive importance of propagule pressure itself varies depending on the situation (Buchan and Padilla 2000). We view the arrival of viable propagules or colonists at an uninvaded site as a critical step of the invasion process and the first of three filters of our framework (Fig. 1). Progress in predicting arrival and propagule pressure on landscapes begins with knowledge of the vectors responsible for the movement of individuals of a species among sites. For example, Johnson and Carlton (1996) reported that aquatic birds play a minor role relative to recreational boaters in the transport of zebra mussels. Further work on recreational boat pathways has found that mussels are most likely to be transported in the adult stage on macrophytes that are entangled on boat trailers (Johnson et al. 2001). For some invasive fishes, bait bucket transport represents the major vector of spread among inland water bodies (Litvak and Mandrak 1993; Ludwig and Leitch 1996), in contrast with natural dispersal through connected waterways. It is clear that knowledge of the vector provides an important starting point for further efforts to characterize the likelihood of species transport to a given site.

Where boating and other human activities are responsible for invasive species transport, simple variables that reflect intensity of human use of lakes such as degree of residential development and the presence of roads and boat launches are easy to collect and have been used as indicators of potential propagule pressure in studies of invasions (Johnson et al. 2009). For example, lakeshore residential development and geographic isolation were significant predictors of invasive rusty crayfish (*Orconectes rusticus*) occurrence in northern Wisconsin lakes (Capelli and Magnuson 1983).

Recent studies have taken a more dynamic approach to modeling invasive species on landscapes (Schneider et al. 1998). Though the spread of invasive species has tradition-

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Table 2. Summary of studies that have used an ecological niche modeling approach to identify locations that are suitable habitat for freshwater invasive species in the Laurentian Great Lakes region.

Studies	Region	No. of lakes	Modeling approach	Comments
Zebra mussel (Dreissena polymorpha)				
Koutnik and Padilla 1994	Wisconsin	194	DFA	Examined relationship between predicted occurrence and environmental variables; did not list suitable sites
Drake and Bossenbroek 2004	United States	—	GARP	Broad-scale predictions from climate and terrestrial variables; no lake-specific predictions
Neary and Leach 1992	Ontario	6000	Environmental thresholds	Based on estimated pH and Ca; generates regional predictions of presence or absence
Strayer 1991	North America	—	Climate match	Regional predictions based on climate; no predictions for specific sites
Spiny water flea (*Bythotrephes longimanus*)				
MacIsaac et al. 2000	North American lakes	49	DFA	Model developed with European database applied to North America
Branstrator et al. 2006	Minnesota	179	DFA	Application of the MacIsaac et al. (2000) model to assess vulnerability of Minnesota lakes
Rainbow smelt (*Osmerus mordax*)				
Mercado-Silva et al. 2006	Wisconsin, Ontario, Maine	5000	CART	Model developed in native range, applied to invaded range; combined with assessment of potential impact
Drake and Lodge 2006	North America	—	GARP	Broad-scale predictions from climate and terrestrial variables; no lake-specific predictions
Smallmouth bass (*Micropterus dolomieu*)				
Vander Zanden et al. 2004*a*	Ontario	3046	ANN	Combined with assessment of road access and potential impact based on food-web structure

Note: DFA, discriminant function analysis; GARP, genetic algorithm; CART, classification and regression tree; ANN, artificial neural network.

ally been modeled as a diffusion process, a more appropriate approach for aquatic invasive species has been to consider the movement of recreational boaters, which comprise the major vector of secondary spread of many aquatic invasive species in the Great Lakes region. Buchan and Padilla (1999) compared a standard diffusion model with patterns of recreational boater activities and found that boater behavior provided better descriptions of the observed pattern of zebra mussel invasions in Wisconsin. The diffusion model underestimated the rate and geographic extent of the invasions because it was unable to incorporate long-distance movements of boaters across unsuitable habitat.

Though detailed boater movement data is often not available, gravity models have been used to estimate the probability of boater movements among sites as a function of boat usage at individual sites and the distance among sites (Schneider et al. 1998). Such models become particularly useful for modeling invasive species spread when they incorporate invasive species life history and dispersal information, as well as the relative positioning of invaded and suitable sites on the landscape. The latter incorporates a critical aspect of invasion dynamics. As new sites are invaded, they themselves can become source populations for further invasions on the landscape (Schneider et al. 1998; MacIsaac et al. 2004; Muirhead and MacIsaac 2005). Thus, propagule pressure at a given site is dependent on the occurrence of the invasive species on the surrounding landscape and would change (presumably increase) over time as the invasion proceeds. Gravity models have proven useful for modeling propagule transport and the risk of species introduction to lakes (Bossenbroek et al. 2001; Leung et al. 2006) and will likely increase in importance as a tool for modeling propagule pressure and the likelihood of aquatic invasive species introductions to new sites on landscapes.

Establishment: can the site support a self-sustaining population?

The second stage considers whether an invasive species, if introduced, would be able to survive and establish a self-sustaining population at a site. Even high levels of propagule pressure of an invasive species at an unsuitable site would fail to result in establishment of a population. Ecological niche modeling has emerged as an approach for modeling suitability of specific sites to biological invaders (Peterson and Vieglais 2001). This approach is based on the concept of the ecological niche as constraining the distribution of a species and is a logical extension of the classical niche concept — that species can occur only in areas that match the ecological conditions to which they are limited. Results of ecological niche modeling can then be projected onto landscapes using a GIS to identify sites inside and outside of the species' niche, thus identifying suitable habitat to a potentially invasive species.

A number of studies have used environmental data to

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identify which inland lakes in the Laurentian Great Lakes region of North America are ecologically suitable for specific aquatic invasive species (Table 2). The simplest approach is to simply consider whether sites have the environmental conditions required for species survival. For example, the zebra mussel requires dissolved Ca concentrations greater than 15 mg·L^{-1} (Sprung 1987; Ramcharan et al. 1992; Mellina and Rasmussen 1994). Provided that dissolved calcium data are available, lakes can be classified as suitable or not suitable based on this threshold value (Vander Zanden et al. 2004*b*). Most studies have used more sophisticated modeling approaches to relate species occurrence to environmental variables. Approaches employed include discriminant analysis, logistic regression, classification and regression tree (CART), artificial neural networks, and genetic algorithm for rule-set production (GARP). GARP is gaining widespread application in forecasting the spread of aquatic invasive species (Drake and Bossenbroek 2004; Iguchi et al. 2004; Chen et al. 2007). The attraction is that predictions can be generated from readily available climate and geology GIS data layers. Applications of GARP to forecasting aquatic invasive species spread to date have not employed site-specific environmental variables (such as pH and dissolved calcium) known to physiologically constrain the distribution of invasive species. Though this approach is useful for scoping the potential distribution of aquatic invasive species at broad spatial scales, it has not yet proven applicable for assessing suitability of specific sites (Drake and Bossenbroek 2004).

Impact: will there be undesired consequences?

The third stage is whether the establishment of an invasive species is predicted to have adverse impacts on the native ecosystem or biota. Impact is the most challenging step of biological invasions to understand and predict (Parker et al. 1999). Invasive species can have a variety of economic or ecological impacts, including declines in native species, habitats, fisheries, and ecosystem goods and services (Parker et al. 1999). Individual invasive species differ widely with regards to their impacts. Many non-native species have established one or more populations but have failed to either spread rapidly or produce measurable adverse impacts (Lodge et al. 1998). Considering that stated management goals are often to minimize the undesired consequences of invasive species (as opposed to simply stopping invasions), these low-impact species should be of lower priority in invasive species management efforts. On the other hand, many invasive species exhibit long time lags in their spread and impact (Crooks 2005), making it difficult to know whether a species has the potential to become problematic in the future.

Although some species appear not to have major adverse impacts, others species are generally considered to have impacts wherever they occur. This is the common assumption for several of the high profile freshwater invasive species of the Great Lakes region, such as rusty crayfish and zebra mussel. Studies of rusty crayfish in the North Temperate Lakes Long-Term Ecological Research (NTL-LTER) lakes in northern Wisconsin found that rusty crayfish reached exceptionally high abundances (20 crayfish·trap^{-1}·day^{-1} in Sparkling Lake and 55 crayfish·trap^{-1}·day^{-1} in Trout Lake), producing dramatic adverse impacts on native aquatic plants, invertebrates, and certain fishes. Wilson (2002) reported adverse impacts on aquatic plants in lakes with catch rates greater than 5 crayfish·trap^{-1}·day^{-1}. Interestingly, a recent survey of 58 lakes in the region found that of the 18 lakes that contained rusty crayfish, only seven (38% of rusty crayfish lakes) had average catch rates that exceeded 5 crayfish·trap^{-1}·day^{-1}, and only one lake had catch rates exceeding 15 crayfish·trap^{-1}·day^{-1} (M.J. Vander Zanden, unpublished data). Thus, the abundance (and, presumably, the impact) of this invader appears to be highly variable among lakes, certainly more heterogeneous than indicated from long-term studies on Sparkling Lake and Trout Lake.

Zebra mussel are another species that are commonly assumed to have major impacts wherever they occur, and only recently has there been explicit consideration of the among-site variation in impact (Ricciardi 2003). In fact, zebra mussel abundance (population density) varies widely among systems (Mellina and Rasmussen 1994; Ramcharan et al. 1992; Strayer 1991). Several measures of ecological impact were found to increase with increasing zebra mussel population density (Ricciardi 2003) such that population density can reasonably be considered a measure of impact. Ramcharan et al. (1992) developed models predicting zebra mussel population density and found that zebra mussel density increased as a function of dissolved calcium concentration and lake trophic status. These findings suggest that using environmental data to classify sites according to potential for adverse impact may be a viable approach (Ricciardi 2003), though it has not yet been widely adopted. Forecasting and habitat suitability studies have generally modeled potential distribution (species presence or absence). The fact that invasive species abundance and impact can vary widely highlights the need to develop a predictive understanding of site attributes that favor high species density and undesired impacts (Byers et al. 2002; Parker et al. 1999; Vander Zanden et al. 2004*a*).

Impact of invasive species can also vary as a function of food-web structure. Vander Zanden et al. (2004*a*) found that the introduction of non-native smallmouth bass (*Micropterus dolomieu*) into Ontario lakes containing pelagic prey fish (such as *Coregonus* spp.) did not affect the forage base of the native top predator, lake trout (*Salvelinus namaycush*). On the other hand, in lakes lacking pelagic prey fish, littoral prey fish are the predominant prey of lake trout. Smallmouth bass introduction dramatically decreases littoral-zone prey fish availability, with negative consequences for lake trout. Thus, the simple presence or absence of pelagic prey fish mediates the impact of smallmouth bass introduction on native lake trout.

Another approach is to consider the value or amenities that the receiving ecosystem provides to humans. Sites or ecosystems that support valuable fisheries and rare and endangered species or are of high recreational value are assumed to experience greater impact if invaded. For example, a study aimed at identifying lakes vulnerable to rainbow smelt invasion considered whether lakes contained fish species previously shown to be negatively impacted by rainbow smelt (Mercado-Silva et al. 2006). Similarly, Schneider et al. (1998) used mussel diversity and the occurrence of threat-

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ened or endangered mussel species as a measure of potential zebra mussel impact on native biota.

Integrating the three stages of species invasion

The conceptual framework presented here (Fig. 1) represents the "scaling-up" of our understanding of biological invasions to the landscape level by viewing a landscape as comprised of many sites that are heterogeneous in terms of their potential for invasive species arrival, establishment, and impact. Though progress has been made in modeling each of the three stages individually (see examples in above section), the power of the approach emerges when assessments at each of the three stages are combined to produce a composite measure of ecosystem vulnerability to an invasive species (Fig. 1). "Vulnerable" ecosystems are those that are most susceptible based on a synthetic and dynamic consideration of propagule introduction, potential for species establishment, and adverse impact (Fig. 1). Studies are now beginning to assess site vulnerability as described here (see below). Notably, these studies tend to find that vulnerable sites comprised a relatively small proportion of all sites on the landscape.

Rainbow smelt in Wisconsin

Rainbow smelt were introduced to Lake Michigan, spread through the Great Lakes, and subsequently spread to inland lakes of the regions, with negative consequences for native species and communities (Evans and Loftus 1987; Hrabik et al. 1998). Mercado-Silva et al. (2006) assessed the vulnerability of lakes in Wisconsin, Ontario, and Maine using this approach. Here, we highlight the three filters of vulnerability for Wisconsin using a lake data set comprised of >5000 Wisconsin lakes (Fig. 2). We assume that the potential for rainbow smelt arrival is based on the presence and number of boat landings at each lake, and that lakes with more than one boat landing are the greatest candidates for introduction. Doing so reduces the number of lakes under consideration from 5000 to 1350 lakes. Environmental suitability (establishment potential) is based on an environmental niche modeling study (Mercado-Silva et al. 2006). CART was used to model rainbow smelt presence or absence using data from their native range in Maine. Application of this model to Wisconsin lakes identified 553 lakes with suitable physical and chemical conditions for rainbow smelt. Finally, potential impacts were assessed based on the presence of five fish species (including species of economic importance) known to be negatively impacted by smelt invasion. Lakes containing one or more species sensitive to smelt introduction were considered candidates for adverse impact (n = 1255 lakes). "Lake vulnerability" is a composite of the three stages of invasion: arrival, establishment, and impact. Of the >5000 Wisconsin lakes considered here, 269 lakes were classified as highly vulnerable to smelt invasion, representing ~5% of the lakes included in the analysis (Mercado-Silva et al. 2006).

Smallmouth bass in Ontario

A second example of this approach is the spread of smallmouth bass in Ontario (Vander Zanden et al. 2004*a*). Smallmouth bass were historically confined to portions of Mississippi and Great Lakes drainages but have gradually been introduced and spread across much of Canada and the US (Rahel 2000). In particular, they have rapidly spread at the northern portion of their native range due to a combination of consistently warm summers during the past two decades, unauthorized introduction by anglers, and accidental bait-bucket transfers (Jackson 2002; Jackson and Mandrak 2002).

Adult smallmouth bass are efficient piscivores and can have substantial predatory impacts on littoral prey fish communities in north-temperate lakes (Vander Zanden et al. 1999). In a study of nine Ontario lake trout lakes, bass invasion was associated with a sharp reduction in fish species richness and abundance in littoral zone habitats. Food-web studies revealed that native lake trout shifted from a diet of littoral fish to one consisting primarily of plankton following bass invasion (Vander Zanden et al. 1999). This food-web change has adversely affected lake trout growth rates, biomass, and productivity.

Considering the tremendous number of lakes in Ontario (>250 000), minimizing the adverse impacts of bass introductions is a daunting task. Vander Zanden et al. (2004*a*) identified a subset of lakes considered vulnerable to bass invasion. The study assessed (*i*) which lakes are likely to receive bass colonists, (*ii*) which lakes are likely to be able to support a viable bass population, and (*iii*) which lakes are likely to be adversely impacted if bass were to establish a population. From this analysis, 30 lakes out of ~800 initial study lakes (<5% of the study lakes) were classified as vulnerable to smallmouth bass invasion. This manageable number of vulnerable lakes would be the obvious focus of management efforts to prevent or slow the further spread of smallmouth bass.

Application to management

Freshwater invasive species are an issue of growing management concern, as local and national government bodies are currently grappling with developing strategies for their prevention, control, and eradication. For example, many US states have recently created invasive species advisory councils that bring together regulators, researchers, and other stakeholders to address research, policy, and management needs (Lodge et al. 2006). Many local and state or provincial governments are currently passing legislation to address the problem of aquatic invasive species and are providing grants for local invasive species control and management efforts. These local granting programs involve selection of invasive species projects for funding, and site vulnerability could be used as a criterion in deciding among invasive species prevention projects. Other strategies for preventing the introduction of invasive species include hiring of watercraft inspectors and (or) educators at boat launches, requiring boats to be cleaned upon entry and exit at boat launches, and even quarantine whereby boats are denied access to certain locations. Such efforts can be costly and even politically controversial and should therefore be directed to the most important locations. In summary, assessment of site vulnerability has the potential to aid in the many aspects of invasive species management currently being implemented at local and regional levels.

In the two examples described above, it was possible to combine the three filters of the invasion processes (Fig. 1)

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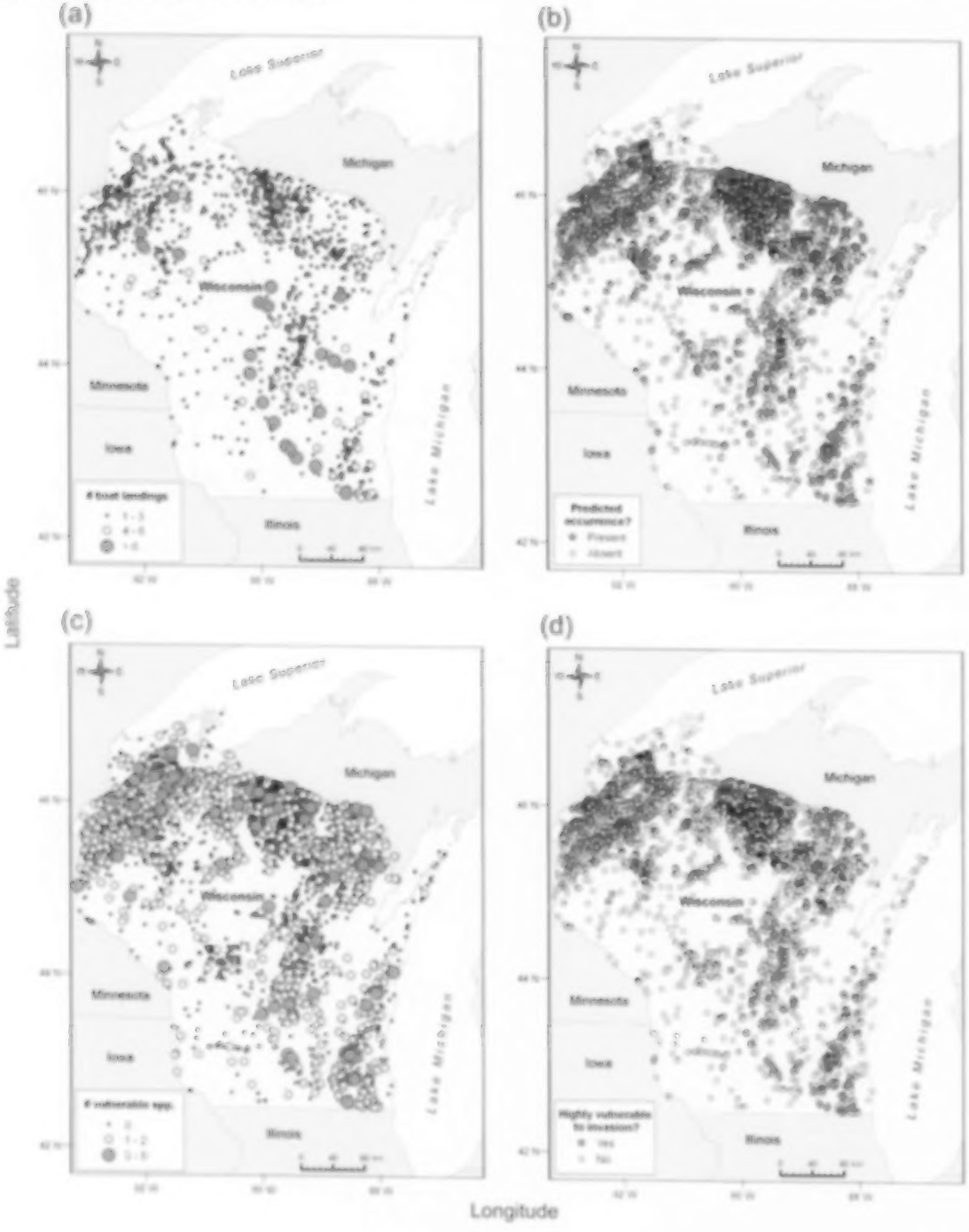
Fig. 2. Assessing lake vulnerability to rainbow smelt invasion for Wisconsin based on consideration of the three filters of a biological invasion: (*a*) introduction potential is based on the number of boat landings; lakes with more than one landing are considered candidates for introduction (n = 1350 lakes); (*b*) establishment potential is derived from environmental niche modeling aimed at identifying lakes with suitable environmental conditions to support rainbow smelt (n = 553 lakes); (*c*) impact potential is based on the occurrence of fish species known to be adversely impacted by rainbow smelt; lakes containing one or more species sensitive to smelt introduction are candidates for impact (n = 1255 lakes); (*d*) vulnerable lakes are those that that are susceptible to arrival, establishment, and unwanted impact and comprise only 269 lakes (Mercado-Silva et al. 2006).



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to assess which lakes on the landscape are most vulnerable to invasion. Notably, a relatively small number of sites were identified as vulnerable to invasion. This makes the approach particularly useful to resource managers because it can help direct interest and concern to a manageable number of sites on the landscape. Yet simply generating lists of vulnerable sites is not sufficient, and the results need to be effectively communicated and made available to end users. Indeed, the concept of "smart prevention" is that knowledge of site vulnerability be used to prioritize and guide invasive species management efforts. For this to occur, it is essential that findings are effectively communicated to resource managers. This will undoubtedly involve developing collaborative relationships between researchers and resource managers. Implementation of this approach can play an important role in bridging the gap between invasive species researchers, resource management agencies, and policy makers (Lodge et al. 2006; McPherson 2004).

One of the important barriers to effective invasive species management programs is that funding for invasive species management and prevention is grossly inadequate. The problem is further confounded by the ineffective allocation of these limited funds. For example, government grants are often made available for invasive species control projects when local citizens become concerned about an invasive species and clamor for action to address the problem. Although invasive species control projects are politically popular, it is generally recognized as a poor use of limited invasive species management resources (Leung et al. 2005; Leung et al. 2002). In the long term, in the absence of prioritizing management efforts around a clearly defined strategy, legislators and the general public will perceive invasive species management programs and expenditures as ineffective and may be unwilling to commit additional resources. On the other hand, thoughtful and carefully targeted programs have the potential to garner broad support because they explicitly attempt to direct resources in the most cost-effective manner.

There are other major challenges involved in preventing or slowing the secondary spread of freshwater invasive species. Although identifying sites that are vulnerable to specific invasive species may improve the allocation of management efforts, it does not provide guidance in allocating effort among different management activities (prevention vs. control) or among different invasive species. Each invasive species will differ with regards to the potential for spread, stage in the invasion process, and responsiveness to prevention and control efforts. Because management funds are chronically limited, effort allocated to one species may be at the expense of dealing with others. This highlights the need to consider the management of any single invasive species within the context of other invasive species. We envision that the general approach presented here could ultimately integrate decision-making options for a suite of invasive species on a landscape. Such efforts should consider not only attributes of the sites and species, but also the extent of spread, the cost and likelihood of success at different sites, and the economic and ecological value of the recipient ecosystems. Certain management actions may simultaneously constrain the spread of multiple species. For example, a small number of lakes may act as invasion "hubs" and should be the focus of efforts to prevent or slow the secondary spread of invasive species (Havel et al. 2005; Muirhead and MacIsaac 2005).

Another challenge involves interactions between biological invasions and other forms of environmental change. The spread and impact of invasive species may be amplified by current trends of land use, climate, and hydrologic alterations (Dukes and Mooney 1999; Mooney and Hobbs 2000). For example, reservoirs may serve as stepping stones for the progression of invasive species across landscapes (Johnson et al. 2009; Havel et al. 2005). Global climate change will fundamentally alter the nature of species interactions in ecosystems with profound and potentially unpredictable implications for the spread and ecological impact of invasive species (Rahel and Olden 2008). Fish species distributions in eastern North America are already shifting northwards as a result of recent warming trends (Jackson and Mandrak 2002), and climate warming is likely to promote the further invasion of fishes into the Laurentian Great Lakes (Mandrak 1989). Finally, invasions may create conditions that facilitate further invasions. For example, the presence of zebra mussels in the Great Lakes is thought to facilitate the subsequent establishment of round goby (*Apollonia melanostomus*; Ricciardi and MacIsaac 2000). Considering interactions among invasive species will become increasingly important as the number and geographic distribution of these species expand.

Conclusions

Invasive species research has been successful in describing the nature and magnitude of the problem. It has been less effective in providing guidance for invasive species management, and science has yet to achieve its full potential in terms of "turning the tide" of biological invasions (Hulme 2006). Though prevention is widely recognized as the cornerstone for successfully managing invasive species, the concept of prevention is multifaceted. Where the identity and basic biology of invasive species is known, recent studies have been successful in identifying vulnerable sites, such that prevention efforts can be targeted to where they are likely to produce the greatest benefit. The approach developed here for the Laurentian Great Lakes region may be transferable to other regions and ecosystem types, particularly where discrete sites or patches can be defined and modeled, such as islands or coastal harbors. A useful parallel is the emergence of the field of reserve design and conservation planning in recent decades. Conservation organizations and resource agencies have undertaken careful planning to set priorities for directing resources so as to best achieve biodiversity conservation goals, leading to more thoughtful allocation of conservation resources. An analogous "conservation planning" perspective can be used to guide efforts to prevent or slow the spread of invasive species. These advances are part of an ongoing transformation of the field of invasion biology from a descriptive science to a normative science capable of improving our ability to manage ecosystems.

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